ERIC F. GREENBERG
ATTORNEY AT LAW

ERIC F. GREENBERG
Of Counsel
Ungaretti & Harris

Contains Confidential Business Information

August 29, 2001

Division of GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St., SW
Washington, DC 20204

Re: NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT, submitted by Pactiv Corporation

To the FDA:

Enclosed is the NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT, submitted by Pactiv Corporation, 1900 West Field Court, Lake Forest, Illinois 60045, c/o the undersigned counsel, consisting of pages 000001.001 through 000250.

Please note that this submission contains Confidential Business Information that Pactiv Corporation desires not to be revealed to Freedom of Information Act requestors and other members of the public. In the first copy of the submission following this letter, the Confidential Business Information has been redacted. For ease of reference, a list indicating which pages contain redactions is attached.

Five complete copies of the submission are enclosed, including the one that has been redacted.

If you have any questions, please contact me at 312 977-4647.

Sincerely,

Eric F. Greenberg
Enc.

000001.001

efgreenberg@uhlaw.com
3500 THREE FIRST NATIONAL PLAZA, CHICAGO, ILLINOIS 60602-4283
PHONE: 312-977-4647 · FAX: 312-977-4405

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Re: NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT

To the FDA:

This letter and its attachments contains the notification, pursuant to the Federal Food, Drug and Cosmetic Act and FDA's regulations, by Pactiv Corporation, 1900 West Field Court, Lake Forest, Illinois 60045, c/o attorney Eric F. Greenberg, 3500 Three First National Plaza, Chicago, Illinois 60602[1], for the General Recognition of Safety of carbon monoxide ("CO") at a level of 0.4% in a modified atmosphere system for packaging fresh meat.

[1] Attachment 1 contains Pactiv's authorization of undersigned counsel, as well as a Summary regarding Pactiv Corporation.

efgreenberg@uhlaw.com
3500 THREE FIRST NATIONAL PLAZA, CHICAGO, ILLINOIS 60602-4283
PHONE: 312-977-4647 · FAX: 312-977-4405

As set forth more fully below and in the attachments to this document, Pactiv believes its intended use of CO is GRAS based on scientific procedures within the meaning of 21 U.S.C. Sec. 201(s) and FDA's implementing regulations in 21 CFR Sec. 170.30, and including FDA's proposed rule published on April 17, 1997 (62 FR 18937). FDA regulations provide that the scientific evidence available and reviewed for a GRAS determination is of the same quantity and quality as that required for a food additive approval, and that the scientific evidence of safety be generally known and accepted by qualified experts in the appropriate scientific and technical fields. 21 CFR Sec. 170.30(a).

I. Claim of Exemption

a. Name and address of the notifier.

Pactiv Corporation
1900 West Field Court
Lake Forest, Illinois 60045
c/o Eric F. Greenberg
Attorney at Law
3500 Three First National Plaza
Chicago, IL 60602

b. Common or usual name of the notified substance.

Carbon monoxide ("CO")

c. Conditions of use (foods, levels, purposes).

When used as described in this Notice, CO meeting appropriate purity specifications is a processing aid in packaging of fresh cuts of muscle meat and ground meat, as a component of a gas mixture utilized in a specific modified atmosphere packaging system. 21 CFR Sec. 170.3(o)(24). A technology utilizing 0.4% CO within a modified atmosphere packaging system will maintain wholesomeness, permit greater flexibility in distribution, and reduce shrinkage, all within a system that results in traditional product display to consumers. All elements of the system, excluding the CO, are already in use in the United States as part of a modified atmosphere meat packaging system called ActiveTech™. Notifier refers to the new system incorporating CO as "AT2001".

Summary

ActiveTech™ is a system that is designed to permit more extended storage of meats, but, as explained below, has no effects on retail display time or characteristics as compared with other modified atmosphere technologies currently in use. It employs materials that are either approved additives used consistently with their approvals, or GRAS substances. AT2001 adapts that system for additional storage scenarios. AT2001 serves to reduce the time needed for enzymatic reduction after modified

atmosphere packaging, and allows consistent display color of whole muscle meats. AT2001's advantages are in the resulting flexibility and consistency during storage and distribution.

The GRAS use of CO described in this Notice involves use as a component of the flush gas mixture used in replacement of ambient air in the packaging for distribution of refrigerated fresh red meat. The meats are in all instances fresh, and are intended to be cooked prior to consumption.

"Traditional" ActiveTech™

The ActiveTech™ modified atmosphere system, in commercial use in the United States since 1998, is a modified atmosphere system for packaging fresh cuts of muscle meat, or portions of ground meat. AT2001 is a refinement of ActiveTech™, and differs from it only in the addition of 0.4% CO to the modified atmosphere.

In the "traditional" ActiveTech™ system, the meats are placed in polystyrene trays and covered with oxygen-permeable, flexible polyvinyl chloride ("PVC") overwraps. The wrapped trays of meat are then placed within an outer barrier bag from which ambient air is removed and replaced with a blend of 30% carbon dioxide (CO_2) and 70% nitrogen (N_2). An activated oxygen-absorbing sachet is also added within the outer bag.

This modified atmosphere maintains the packaged meat in an oxygen-free deoxymyoglobin state, with its distinctive purplish appearance that is generally considered undesirable by consumers. The traditional ActiveTech™ system relies on the rapid reduction of the oxygen content of the outer bag. Once the oxygen is removed, a "seasoning" phase begins during which enzymatic effects take place so that the meat will be able to "re-bloom" when once again in the presence of oxygen. As the residual oxygen in the package is consumed by the activated oxygen scavenger, red meat oxymyoglobin is first subject to rapid conversion to metmyoglobin (brown) at very low partial pressures of oxygen, e.g. 0.5% oxygen. This low partial pressure region of oxygen is necessarily passed through prior to ultimately reaching 0% in the package and the conversion to deoxymyoglobin (purple). This seasoning phase can take up to 5 days.

Before display to consumers at retail, the outer bag, and thus the modified atmosphere, is removed, and the traditionally wrapped product (in polystyrene foam tray with PVC overwrap) is permitted to "re-bloom" to its familiar appearance through creation of oxymyoglobin on the meat's surface.

AT2001

In the AT2001 modified atmosphere system, as with traditional ActiveTech™, fresh cuts of muscle meat, or portions of ground meat, are placed in polystyrene trays and covered with oxygen permeable flexible PVC overwraps. The wrapped trays are placed within the outer barrier bag, the air is removed and replaced with a blend of 0.4% CO, 30% carbon dioxide (CO_2) and the balance nitrogen (N_2). As with the traditional AT system, an activated oxygen-absorbing sachet is added within the outer bag to create and maintain an oxygen-free environment for the packaged meat during storage.

As noted, meat packaged in traditional ActiveTech™ undergoes a myoglobin pigment conversion from oxymyoglobin (red) to metmyoglobin (brown) to deoxymyoglobin (purple) in the oxygen free environment. The metmyoglobin formed generally will convert to deoxymyoglobin in the oxygen free storage environment in about 5 days, a period of time referred to as the "seasoning period". However, the meat's ability to convert all of the metmyoglobin formed to deoxymyoglobin during the seasoning period and then fully rebloom to oxymyoglobin upon re-exposure to normal, oxygen-rich atmosphere at retail, is a function of a multitude of unpredictable, uncontrollable factors in the meat such as age, muscle

location, and enzyme energy level. This is a key weakness of all current low oxygen packaging systems.

Meat packaged in the AT2001 atmosphere will instead convert from oxymyoglobin to carboxymyoglobin (red) in the package due to the inclusion of 0.4% CO in the modified atmosphere. This conversion occurs during the initial 24 hours as the free oxygen in the headspace is consumed. Thus, the CO effectively protects the myoglobin from converting to metmyoglobin as the oxygen in the package is removed. This feature is especially important for the most pigment sensitive meats such as those from the round. The meat will continue to maintain its red color while in storage until the package is opened for retail display, when the outer bag (and modified atmosphere) is removed. Since carboxymyoglobin and oxymyoglobin are essentially the same colors, no seasoning period is necessary. The meat can be opened for retail display the day following packaging.

Once in retail display, the meat's myoglobin will begin the rather slow, natural conversion to metmyoglobin (brown), akin to that seen with untreated meat, allowing for a conventional retail display life of 3 to 4 days, closer to consumers' expectations of color than results from use of high

oxygen packaging systems. Attachment 2 consists of photographs depicting the ActiveTech™ and AT2001 systems.

The AT2001 formulation will assure that the meat will have the familiar color during and following storage, eliminating the seasoning period, allowing for placement in retail display beginning at 1 day, and up to 30-40 days, after packing. For cuts of meat from the round, and other color sensitive cuts, the AT2001 will help them have a more uniform red color for retail display.

In AT2001 (as in traditional ActiveTech™), the trays and films utilized are made from familiar, FDA-approved polymers that are used in accordance with their existing approvals or GRAS status. The activated oxygen-absorbing sachet inserted into the outer bag to absorb oxygen does not contact food and is not expected to become a component of the food. Therefore, it is not a food additive under the definition in 21 USC Sec. 321(s). As an added assurance of safety, each of the sachet's components has some GRAS status or food-related approvals.

Thus, the AT2001 system adds a refinement to the existing ActiveTech™ system that will allow its utilization with whole and ground meat products that meet the processors' desire to get to market as soon as the day following processing.

d. Basis of GRAS determination: Scientific procedures

CO safety

Pactiv believes its proposed use of CO is GRAS based on scientific procedures within the meaning of 21 U.S.C. Sec. 201(s) and FDA's implementing regulations in 21 CFR Sec. 170.30 and including FDA's proposed rule published on April 17, 1997 (62 FR 18937).

CO is a colorless, odorless gas that is poisonous to humans if inhaled at much higher levels than are involved with the use that is the subject of this Notice. It is formed when carbon is not completely burned, for example, in the combustion of fuels.

It is well known that CO creates negative health effects at elevated levels because it out-competes oxygen for attachment to the hemoglobin molecule. The resulting carboxyhemoglobin levels in the blood are associated with severe health effects. In addition, the equilibrium rate for the exchange of carboxyhemoglobin for oxyhemoglobin is very slow, and the resulting level of carboxyhemoglobin is a function of the CO level in the respired air, the time of exposure and the level of activity of the individual. Typical atmospheric CO levels are <20 mg/m^3 as an 8 hour mean (higher in

urban and high traffic areas), and typical carboxyhemoglobin levels due to natural background CO range between 1.2 and 1.5%.

CO is recognized as a significant air pollutant at higher levels. Automobile exhausts, industrial processes and boilers and incinerators all contribute to air quantities of CO. According to the U.S. EPA Office of Air and Radiation:

> Carbon monoxide enters the blood stream and reduces oxygen delivery to the body's organs and tissues. The health threat from exposure to CO is most serious for those who suffer from cardiovascular disease. Healthy individuals are also affected, but only at higher levels of exposure. Exposure to elevated CO levels is associated with visual impairment, reduced work capacity, reduced manual dexterity, poor learning ability, and difficulty in performing complex tasks. EPA's health-based national air quality standard for CO is 9 parts per million (ppm) [10 mg/m^3] measured as an annual second-maximum 8-hour average concentration.

"Summary regarding carbon monoxide" as part of discussions of 6 principal pollutants, U.S. EPA Office of Air and Radiation.

No health effects result when carboxyhemoglobin levels are under 4% to 5% in healthy adults. Carboxyhemoglobin levels of 2 to 3% may have negative effects on those with cardiovascular disease or other sensitivity. See, Environmental Health Criteria 13, Carbon Monoxide, World Health Organization, Geneva, Switzerland (1979), p. 15.

The US Occupational Safety and Health Administration's air contaminants regulation, 29 CFR Sec. 1910.1000, lists 50 ppm and approximately 55 mg/m^3 of CO as the 8-hour Time Weighted Average of exposure for the substance. 29 CFR Sec. 1910.1000.

By contrast, as explained below, the worst case estimated intake of CO attributable to AT2001 is 1.88 mg CO/meal.

The US FDA has not established an Acceptable Daily Intake for CO. Nevertheless, CO exposure, at levels much higher than those attributable to AT2001, for decades has been permitted within the existing FDA and USDA food additive regulatory provisions:

- Wood smoke ("smoke flavoring"), conventionally including CO as a component, is permitted by regulation as an ingredient in meat and poultry products pursuant to 9 CFR Secs. 318.7(c)(4)[meat], 381.147(c)(4)[poultry], 424.21(c).
- The specifications for Combustion product gas in 21 CFR Sec. 173.350 permit CO up to 4.5 percent by volume in such gases, which may be used in the processing and packaging of beverages and other foods except fresh meats, to remove and displace oxygen. Such gases are commonly used to package fruits and vegetables.

In 2000, FDA responded favorably to GRAS Notice 000015 from Hawaii International Seafood, Inc. for the use of tasteless smoke, before freezing of tuna, as a preservative, specifically to preserve taste, aroma, texture and color. GRAS Notice (GRN) No. 000015, March 10, 2000. CO is a primary component of conventional smoke, which that Notice asserts is Generally Recognized As Safe based on decades of safe use in a variety of foods, which uses are recognized by FDA and incorporated into numerous food standards that permit smoking of cheeses. CO is also a primary component of tasteless smoke, along with nitrogen, oxygen, carbon dioxide and methane. The tasteless smoke is used to impart a "preservative effect." As noted in FDA's March 10, 2000 letter about the GRAS Notice, "In Hawaii International's view, tuna treated with tasteless smoke and tuna treated with conventional smoke contain comparable levels of carbon monoxide, carbon dioxide, hydrocarbons, and phenols." FDA's letter notes that it "has no questions at this time" regarding Hawaii International's conclusion that the use described is GRAS, though, in keeping with current regulatory practice, it had not made its own determination.

CO is listed as a reproductive toxicant by the State of California pursuant to its Safe Drinking Water and Toxic Enforcement Act of 1986 ("Proposition 65"). California law contemplates that exposures to listed reproductive toxicants will be accompanied by a warning, unless the exposure is less than 1/1000th of an established no observable effect level. Cal. Health and Safety Code, Sec. 25249.6. No such level has been established for CO. Almost without question, though, any such future level (which will have a dubious connection to safety principles in any event, due to the design of Prop. 65), will be more than 1,000 times any possible exposure that could result from AT2001. The worst-case potential exposures from AT2001 are tiny fractions of the established occupational and environmental exposure levels (see below), which themselves are certain to be well below any level at which reproductive toxicity is ultimately is deemed to result.

Effects on Fresh Meat and Consumption

Analysis of the AT2001 system makes plain the lack of any safety issue from consumption of treated meats. Additionally, similar technologies employing CO as part of a modified atmosphere gas mixture analyzed the technologies for effects on meat in terms of microbial load and organoleptic

properties including color, and for the safety of consumption of treated meats, specifically, any tendency of the consumed meat to expose consumers to levels of carboxymyoglobin. Further important evidence is obtained from examination of the actual experience since 1985 in Norway of packaging fresh red meats in 0.3 – 0.5 % CO for retail.

Safety: Effects on carboxymyoglobin levels

Consumption of meat treated with AT2001 is not expected to result in any measurable levels of carboxymyoglobin in the blood of those who consume treated meat.

An Estimated Daily Intake ("EDI") of CO attributable to the AT2001 use can be calculated as follows. First, we assume the following reasonable values for the exposure parameters:

(1) An AT2001 bag contains 1.5 L modified atmosphere with a CO concentration of 0.4%, that is equivalent to approximately 0.006 L of CO in the bag (= 6 mL CO).

(2) At 28 g CO per mole and approximately 22.4 L per mole, the mass of CO per unit volume may be calculated: (28 g/mol)/(22.4 L/mol) = 1.25 g/L = 1.25 mg/mL.

(3) The AT2001 bag contains 2 lbs (approximately 1.0 kg) of ground meat.

(4) Approximately 30% of the total amount of CO is absorbed into the meat (based on Watts, D.A.; Wolfe, S.K.; Brown, W.D., "Fate of [^{14}C] Carbon Monoxide in Cooked or Store Ground Beef Samples", J. Agric. Food Chem., Vol. 26, No. 1 (1978), pp. 210-214). Therefore, the amount of CO taken up by the meat is [(0.3)*(6 mL/bag)*(1.25 mg/mL)] / [1.0 kg meat/bag] = 2.25 mg CO / kg meat.

(5) If we assume that a person consumes an 8.8 oz steak (250 g = 0.25 kg meat), or ground equivalent, at a single meal[2], that 85% reduction in CO content occurs during cooking, and that 100% of the ingested CO is absorbed, then the maximum amount of CO exposure is (0.15)*(2.2.5 mg CO /kg meat)*(0.25 kg meat/meal) = 0.084 mg CO/meal.

Next, comparison may be made of the of the consumer EDI for CO to that amount inhaled during an 8-hour period at the EPA's National Ambient Air Quality Standard ("NAAQS") level. 40 CFR Sec. 50.8, National primary ambient air quality standards for carbon monoxide:

[2] Note that this is a conservative assumption. The EDI of beef for the 90th percentile intake per user is 139.2 g/d based on the most recent USDA national survey of food intake by individuals. Pactiv chose to use a larger value for beef consumption to simulate a typical to above-average consumption incident rather than an average over all meats.

The calculated, worst case consumer EDI for CO may be compared to that amount inhaled during an 8-hour period at the American Conference of Governmental Industrial Hygienists ("ACGIH") Threshold Limit Value ("TLV"). Documentation of the Threshold Limit Values and Biological Exposure Indices, p. 23, ACGIH, 1330 Kemper Meadow Drive, Cincinnati, Ohio.

(1) The ACGIH TLV is 25 ppm CO is equivalent to approximately 28.9 mg CO per m^3 air.

(2) The typical person breathes 15 m^3 air per day or approximately 5 m^3 air per 8-hours.

(3) The exposure under these circumstances may be calculated as follows:

$$(28.9\ mg/m^3)*(5\ m^3/8\text{-}hr) = 145\ mg\ CO\ /\ 8\text{-}hr.$$

Thus, the ingestion of residual CO from the cooked meat is merely 1.3% of the exposure level at ACGIH TLV ((1.88 mg) / (145 mg) = 0.013 = 1.3%)

Finally, the calculated worst case consumer EDI for CO may be compared to that amount inhaled during an 8-hour period at the OSHA PEL:

(1) The OSHA PEL is 50 ppm CO is equivalent to approximately 58 mg CO per m^3 air.

(2) The typical person breathes 15 m^3 air per day or approximately 5 m^3 air per 8-hours.

(3) The exposure under these circumstances may be calculated as follows:

$$(58\ mg/m^3)*(5\ m^3/8\text{-}hr) = 290\ mg\ CO / 8\text{-}hr.$$

Thus, the ingestion of residual CO from the cooked meat is 0.65% of the exposure level at OSHA PEL. ((1.88 mg) / (290 mg) = 0.0065 = 0.65%).

Thus, the consumer EDI of CO from a eating meat packaged in the Active Tech 2001 bag is a small fraction of any of the currently allowed exposures by authoritative agencies. As these various limits were established to protect individual safety and health, it is plain that the worst case exposures that may result from AT2001 present no safety concerns whatsoever.

In the 1997 study, "Technological, hygienic and toxicological aspects of carbon monoxide used in modified-atmosphere packaging of meat" Trends in Food Science and Technology, September 1997 [Vol. 8], pp. 307-312, Sørheim, et al. concluded that meat packaged and displayed in an atmosphere combining 60 to 70% carbon dioxide, 30 to 40% nitrogen, and less than 0.5% CO "will result in only negligible levels of carboxyhemoglobin in the blood."

The authors note that there was sparse information in published literature on exposure to CO after consumption of meat treated with CO gas. They note that "the inhalation of air containing CO at a level of 55 mg per m^3 (the acceptable level in working environments in the USA) would provide a COHb level for a prolonged time period (hours) of at least 14 times that of the level reached temporarily on the consumption of 225 g of meat that has been packaged in CO at the saturation level for myoglobin." That estimate assumed saturation of meat myoglobin and hemoglobin was maximal and the transfer of CO from the gastrointestinal tract to the blood was 100%. Sørheim, et al. (1997), p. 310. The authors concluded, "Consequently, even for such a "worst case" scenario, the treatment of meat with CO gas appears to contribute very little to COHb levels, relative to levels that are considered safe in the working environment." Sørheim, et al. (1997), p. 310.

The authors report that "CO is lost from previously CO-treated meat during storage in the absence of CO, with a half life of ~3d." Sørheim, et al. (1997), p. 310. As these fresh meats are to be cooked before consumption, CO lost during cooking is also relevant. The authors report that "When the beef was cooked at 195° C, only 0.1 mg of CO remained

per kg of meat. The loss of CO amounted to ~85%." Sorheim, et al. (1997), p. 310.

The authors also compared CO exposure from the air and estimated exposure from CO-treated meat. Their comparative table is shown below.

Table 5. Theoretical Uptake of Carbon Monoxide (CO) in Blood

Exposure method	**CO intake in 1 h**	**CO intake in 8 h**
Lungs ($15m^3$)	24 mg x 0.625 = 15.1 mg	9.2 mg x 5 = 46.0 mg
Meat (250 g CO treated)	0.025 mg	0.025 mg

Sørheim, et al. (1997), p. 311, Table 5.

Part of the authors' analysis was the premise that absorption of CO from the gastrointestinal tract into blood will in all probability be less effective than absorption from the lungs. The authors summarized the comparison as follows:

> In order to prevent a maximum COHb level in the blood of 1.5% being exceeded, the CO concentration in air for a 1h period of moderate physical activity should not exceed 24 mg/m^3, or 9.2 mg/m^3 in 8h (according to Table 4). In contrast, the consumption of meat that had been treated for 3d in an atmosphere containing 1% CO yielded ~0.1 mg of CO per kg of meat on storage and cooking.

Sørheim, et al. (1997), p. 310, citing Watts, D.A.; Wolfe, S.K.; Brown, W.D., "Fate of [^{14}C]Carbon Monoxide in Cooked or Stored Ground Beef Samples", *J. Agric. Food Chem.*, Vol 26, No. 1 (1978), pp. 210-214.

The authors calculate that CO intake in 1h through the lungs taking in 15m^3 per day would result in 15.1 mg of CO, as compared with 0.025 mg of CO from intake of 250 g of CO treated meat. In 8 hours, the authors say the lungs will take in 46.0 mg, and the figure for meat would still be 0.025 mg. As the authors conclude,

> Estimates detailed above indicate that, even assuming an improbable 100% absorption of CO from the gastrointestinal tract into the blood, the consumption of meat that has been treated with 1% CO will result in COHb levels that are negligible (approximately 3 orders of magnitude lower) compared with those resulting from exposure in the working environment to CO at an acceptable level. Consequently, it is highly improbable that CO exposure from meat packaged in an atmosphere containing up to 0.5% will represent a toxic threat to consumers through the formation of COHb.

Sørheim, et al. (1997), p. 310.

In another published report, the storage life and characteristics of meats packaged in a modified atmosphere including 0.4% CO were studied, but under circumstances distinguishable from AT2001. Sørheim O; Nissen, H; Nesbakken, T, "The Storage Life of Beef and Pork Packaged in an Atmosphere With Low Carbon Monoxide and High Carbon Dioxide", 52 Meat Science 157-164 (1999). In the study, the meats were packed in

modified atmosphere into retail-ready packages. This study examined off odor and microflora, as well as color, comparing the 0.4% CO/ 60% CO_2 /40% N2 gas mixture with a gas mixture of 70% oxygen and 30% CO_2.

Among the points made by these authors was that there is sometimes an objection raised against using CO in retail ready meats because "the colour stability can exceed the microbiological shelf life, with the risk of masking spoilage of the meat." Sørheim, et al. (1999), p. 163. (Citing Kropf, D.H. (1980), "Effects of retail display conditions on meat colour", *Proceedings of the Reciprocal Meat Conference, 33, pp. 15-32.*) The authors assert that in those circumstances, consumers would need to rely on off odors to evaluate microbiological conditions of meat. In addition, they caution, "When a MA with CO is applied commercially, it is important to have a proper control of hygienic condition of the meat raw materials and the chill chain temperatures." See Sørheim, et al. (1999), p. 163.

AT2001, by contrast, presents no such similar problems or needs for caution. AT2001 does not mask spoilage of the meat. AT2001 does not involve use of a modified atmosphere including CO in the retail package. Moreover, as noted below, Pactiv's own commissioned experimentation with AT2001 demonstrates that AT2001 retail packages will deteriorate in color beginning almost immediately after removal of the modified

atmosphere, and that microbial load will not reach unsafe levels while the color of AT2001 meat is still acceptable to the consumer.

Safety: The Norwegian experience

In Norway, CO has been used to package fresh meats, even at retail, since 1985, with commercially satisfactory and safe results.

The 2000 submission by the Norwegian Meat Cooperative and Norwegian Independent Meat Association to the EU Commission seeking Europe-wide approval of the use of CO, "Application For Assessment Of The Food Additive Carbon Monoxide (CO) Prior To Its Authorization", is Attachment 3. The evaluation undertakes a detailed analysis of the CO exposure expected through the described packaging use. See section entitled "IV. Report by Tore Aune: "Fresh Meat in Consumer Packaging-A Toxicological Evaluation of the Use of Up to 0.5% CO in a Gas Mixture".

As the Norwegian risk assessment analysis concludes, assuming a worst-case exposure of 0.1 mg/kg from consumption of 250 grams of heated CO-treated meat, CO intake can be expected to be 0.025 mg in 1 hour or even after 8 hours. Attachment 3, p. 000154. The cited study, Sørheim, et al. (1997), utilized meat that had been treated with 1% CO. According to the authors, to stay under maximum blood levels of carboxyhemoglobin of 1.5%, "the CO concentration in the air must be 24

milligrams per mg/m^3 for 1 hour at moderate physical activity at 9.2 mg/m^3 for 8 hours...." Attachment 3, p. 000154. Assuming an adult inhales 15 m^3 per 24 hours, this translates to 15.1 mg of CO taken in 1 hour, or 46.0 mg of CO taken in 8 hours. This is in dramatic contrast to the miniscule amount expected to be ingested through meat. The Norwegian authors conclude, "From a health perspective, the use of CO in concentrations below 0.5-1% for fresh meat thus represents no toxicological risk." Attachment 3, p. 000155.

Safety: Exposure in environment

As a basis for comparison, the possible effect on ambient CO concentration associated with the release from a typical AT2001 barrier bag was estimated. A typical AT2001 barrier bag contains approximately 1.5 liters of modified atmosphere with a CO concentration of 0.4 percent, which is equivalent to approximately 0.006 liters of CO within the bag. On a mass basis, this volume of CO is equivalent to approximately 0.0075 g (7.5 mg) CO per bag.

Consider the possible use of the bag for storage of meat prior to retail display (e.g., at a supermarket). Any unassociated CO within the bag, it can be assumed, would be released to the meat processing area when the bag is opened, resulting in possible exposure by the employee(s) to the

released CO. The extent of such exposure is dependent on several factors, including the size of the meat processing area, air-mixing within the area and between adjacent areas, the number of bags opened, and the amount of free CO unassociated with the meat in the package. For these calculations, it has been conservatively assumed that none of the CO has become associated with the meat and is therefore all free to the ambient atmosphere upon opening of the package.

Assume, however, that the air volume within a meat processing area may reasonably range from 150 m^3 to 1,500 m^3, which would represent several hundred to several thousand square feet of processing area. If each bag introduces 7.5 mg CO to the air within the processing area, the corresponding concentration of CO in air would be in the range of 0.005 mg/m^3 to 0.05 mg/m^3, assuming conservatively that there is no air exchange between the processing area and other rooms or the outdoors. Thus, to exceed the occupational safety standard (i.e., threshold limit value, or TLV) of 25 ppm (29 mg/m^3), 580 to 5,800 1.5 liter bags would need to be opened within an 8-hour period. As noted above, this assumes no mixing with other areas of the building or with outdoor air.

Thus, applying the reasonable assumption that the air volume within the processing area will be exchanged with external air once per hour,

opening of 580 to 5,800 bags per hour would be required to exceed the TLV, or 4,600 to 46,000 bags per work day. The number of bags opened within a given processing area will be a function of the size of the processing area, to a given extent, but is unlikely to even approach the number of bags required to result in air concentrations at the TLV. Actual concentrations in the work area of a secondary processing facility would likely be one to two orders of magnitude below the standard. Thus, opening of bags within a work area will not alter significantly the environmental exposure to CO.[3]

Regardless, the opening of the bags does not alter significantly the environmental exposure to CO. This action qualifies for a categorical exclusion from preparation of an environmental assessment pursuant to 21 CFR Sec. 25.32 (i), which provides an exemption for, in pertinent part, "Approval of a ...GRAS affirmation petition...." 21 CFR Sec. 25.32(i). The regulation makes no specific mention of the GRAS Notice procedure, but similar treatment is warranted for a GRAS Notice. (We also note that CO as used here also qualifies for exclusion under 21 CFR Sec. 25.32(r), as CO "occurs naturally in the environment" and the noticed use "does not alter significantly the concentration or distribution of the substance, its

[3] As an aside, there is no reason to expect any difficulty achieving compliance with the OSHA Threshold

metabolites, or degradation products in the environment." 21 CFR Sec. 25.32(r).)

Corroborative information about AT2001

The specific AT2001 system has been thoroughly tested to confirm that it results in the expected limited exposures to CO, and has no adverse effects on the treated meats. A study of meats treated with AT2001 commissioned by Pactiv examined its effects on initial product color, stability of color during display, and the central safety consideration of the relationships between color deterioration and microbial populations.

The study, conducted by faculty of the Department of Animal Sciences & Industry of Kansas State University, Manhattan, Kansas, examined steaks from three cuts of beef (strip loin, tenderloin, and inside round steaks), as well as ground beef. The study report is Attachment 4. The meats were packaged in AT2001 atmosphere, then stored at 35° F or 43° F for up to 35 days. Cuts were then placed under simulated retail conditions by being removed from the AT outer package and displayed at 34° F until their color approached consumer unacceptability. Comparisons

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Limit Value at plants using the AT2000 technology to fill bags. Experimental use of an exhaust hood over the machinery has resulted in no measurable increase in CO ppm levels near the line.

were made to similar products that had been exposed to oxygen but not CO.

Among the study's conclusions were:

(1) Color: AT2001 *system resulted in products that were equally red to products packaged with traditional oxygen permeable overwrap.* When the AT2001 outer bag was removed, the product's conversion to oxymyoglobin occur red in 60-90 minutes and then had a typical bright red color. Visual appearance was improved, especially in the tenderloin and inner part of the inside round steaks, throughout display. Color deterioration compared well to baseline products exposed to oxygen. For tenderloin and inside round steaks, and to a lesser degree for ground beef, display time was increased only slightly in the AT2001 samples.

(2) Bacterial growth: Bacterial growth was neither encouraged nor suppressed by the addition of CO to the ActiveTech™ *gas blend (nitrogen and carbon dioxide)*, although microbial growth curves changed in slope and exponential growth according to the environment in the packages. Aerobic bacteria and facultative anaerobes followed typical patterns of growth according to environmental conditions.

(3) Spoilage indicators: CO neither masked spoilage, nor extended color life beyond the point of wholesomeness (i.e., the point of microbial soundness).

A summary of the study follows.

A random selection of all steaks and ground beef packaged using oxygen-permeable polyvinyl chloride ("PVC") film were placed in display to serve as a baseline for color and microbiological comparisons. Products were expected to have the lowest microbiological load and ideal color stability using traditional packaging and display conditions for products exposed only to atmospheric oxygen. The inherent muscle chemistry responsible for good color life also was optimal. If the product exposed to CO were to have extended meat color life, then it will be compared to the baseline "control" with the "best" possible color.

To measure color changes, visual scores were considered the "standard" with instrumental color being discussed relative to its agreement or disagreement with the visual panel, ie, did the objective measurements confirm what the color panel saw? Visual scores of ≥ 3.5 were considered borderline acceptable. When samples reached this discoloration, they were removed from display. Normally, a* values (higher values indicate more redness) are highly correlated to visual appraisal.

Inside round steaks typically are two-toned in color. The inner portion (ISM) is much less color stable than the outer portion (OSM). These portions were scored separately since one portion may have acceptable color while the other has unacceptable color that would be discriminated against by consumers resulting in the whole cut being judged unacceptable in color. The effects of CO on this bi-colored muscle were needed to confirm that color was not excessively extended in either portion.

Average fat and moisture contents of the ground beef were 19.5 and 61.6%, respectively. The pH of both intact muscles and the ground beef ranged from 5.3 to 5.7. The initial aerobic plate counts and lactic bacteria counts for all products were relatively low and indicative of good microbial quality of the raw materials and good sanitation. Furthermore, coliforms and *E. coli* were below the detection limit throughout the study.

The color of ground beef and steaks entering display (after MAP storage at 2 temperatures) was an attractive, typical red color. Although there were several significant differences in visual scores and a* values (Attachment 9, Table 2 and Figures 1-10 at day 0) for product CO vs. baseline cuts, the variation in color was usually within $\pm$ 0.5 of a color score.

Color results: In general, the initial color of product exposed to CO was very similar to the color of steaks from the baseline display (never exposed to CO). When differences occurred, they were more related to either storage temperature or postmortem age of the product.

Panelists did not consider the color of product exposed to CO atypical. Cuts exposed to CO generally appeared more uniformly bright-red and would be expected to have high consumer appeal. These results were expected, as CO is known to preferentially form a ligand with the colored pigment (myoglobin) in meat resulting in a more intense red pigment known as carboxymyoglobin.

In the AT2001 system, Pactiv uses a low level of 0.4% CO, and obtains a red color very similar to the normal red oxymyoglobin pigment of fresh meats exposed to oxygen.

Color stability results: A critical next question was whether the carboxymyoglobin formed on the surface was more stable than the oxymyoglobin formed in baseline product. Further, did the carboxymyoglobin deteriorate in a predictable way that consumers could continue to use visual color to judge freshness or potential spoilage?

Product exposed to CO during MAP storage had color deterioration during display. (See visual panel scores (Attachment 4, Figures 1-5) and

instrumental color (a* values, Attachment 4, Figures 6-10).) As expected, visual scores increased (color deteriorated) and a* values decreased (loss of redness) as days in display increased. In several instances, color appeared to improve late in display – as indicated by a decrease in visual scores (see ground beef, strips loins and tenderloins at 43°F). These decreases were not a return of redness, but resulted from removal of discolored packages the preceding period, leaving product with less overall discoloration remaining in the case.

In general, the color deterioration profiles followed an expected pattern. Namely, the freshest product (baseline packages) had the most stable, red color and the most days in display needed to reach borderline discoloration of all treatments. (Attachment 4, Table 3 scores to 3.5) Exceptions occurred for the inside portion of the inside round and tenderloin products, where the product exposed to CO had slightly more stable color than the baseline product (Attachment 4, Table 3). These two muscle areas are well known by retailers as having short color life. Thus, CO appeared to improve color life when the inherent muscle chemistry desired for color was limited.

For product from MAP, the longer the storage time, the faster the deterioration, especially at the higher storage temperature (Attachment 4,

Tables 2 and 3). For packages stored at 43°F, which was a mildly abusive temperature, color deterioration would be expected to accelerate. This phenomenon also is illustrated in Attachment 4, Figures 1-10.

There was no evidence the color shelf life was unexpectedly lengthened by exposure of meat to CO in MAP. Changes in a* values (and other instrumental measures of color not shown) followed the same pattern of color deterioration observed by the visual panelists.

Color and microbial data: Initial, pre-display microbiological data suggested that the raw materials were fresh and processed using good hygienic practices. For intact cuts, lactic acid bacteria, generic *E. coli*, and total coliform counts were below the detection limit of 1.76 colony forming units (CFU)/in^2. Initial, pre-display aerobic plate counts ("APC") for intact muscles ranged from 1 to 1.63 log_{10} CFU/in^2 . Post-display counts were higher ($P<0.05$) than pre-display APC which was an increase in bacterial proliferation and typical deterioration. However, all product had sufficient microbes to be susceptible to spoilage.

Baseline products were pulled from display when the visual panel scores reached ≥ 3.5. However, the APC did not exceed 5 log_{10} CFU/unit as shown in Attachment 4, Figures 15-18. Furthermore, off-odor scores for

product at end of display (Attachment 4, Table 3) ranged from no to slight off odor.

Thus, color life in this base population did not exceed microbial soundness, which is generally accepted as < 100 million CFU/g hamburger (<1 x 10^8). (Principles of Meat Science, 3d Ed., Hedrick, H.B.; Aberle, ED, Forrest, JD; Judge, MD; Merkel, RA, Eds, Kendall/Hunt Publishing Co., Dubuque, Iowa.

Similar trends in microbial growth occurred in post-displayed samples stored in MAP compared to baseline products. Microbial patterns for product deterioration are shown in Attachment 4, Table 4 and Figures 11-18. Products stored under MAP at a slightly abusive temperature showed, as expected, a more rapid increase ($P<0.05$) in microbial counts compared to samples stored at 35°F. For post-MAP (pre-display) and post-display samples, APC were higher at 45°F than 35°F (Table 4), and during the later days of storage at the higher temperature, differences were more obvious. Significant changes ($P<0.05$) occurred in all cuts and ground beef with the exception of semimembranosus muscle. Counts for the SM muscle were lower than expected and no significant changes occurring until day 35 of MAP storage. This suggests that quality products that have been handled

in a sanitary fashion can be stored in the AT2001 system up to 35 days without comprising microbial quality.

The APCs for intact strip loin and tenderloin steaks stored at 35°F were lower ($P<0.05$) on all days of display on days 21 and 35 post-MAP than steaks stored at 43°F (Attachment 4, Figures 12 and 14). Although products did not show a difference in APCs 7 days post-MAP, those products stored at the higher temperature (43°F) were more inferior 21 and 35 days post-MAP.

One goal of this research was to see if the color of CO-treated meat might mask spoilage. Visual color scoring was considered as the "standard" for determining the time to remove products from display. Because the visual panel scores were the deciding factor for length of shelf life, the interdependence between visual color and APC, LAB, and odor were considered quite important.

Attachment 4, Figures 19-21 show aerobic and lactic bacterial growth and odor scores at the end of display plotted against their corresponding visual color scores. All data observations were summed over storage temperature, storage time, and product type and plotted in one graph. If color masked spoilage, then there would be multiple points in the upper left

quadrant of the plot, the area represented by unacceptable microbial counts and off odors but with acceptable color (i.e., scores <3.5).

This did not occur with any frequency in any of the three plots. Thus, it does not appear that exposure of meat to CO during extended (up to 35 days at either 35° or 43°F) caused meat color to hide spoilage.

e. Statement of availability of information

Notifier has relied on published studies and generally accepted scientific data and information as the basis of its conclusions, and those of its panel of experts, about the safety and the general recognition of a modified atmosphere system for meat incorporating 0.4% CO in the gas mixture.

II. Identity of notified substance

1. Chemical name: Carbon monoxide

2. Chemical Abstracts Service: **630-08-0**

3. Composition Specifications for food-grade material: The CO employed in this system is to be of suitable purity for food contact. Specifically, this will mean a 99.99% minimum purity, as supplied by Pactiv's commercial gas supplier, Haun Welding Supply, Inc., 6481 Ridings

Road, Syracuse, NY 13206. Attachment 5. The supplier's CO meets the following specifications, and will be referred to as "commercial grade":

Component	Specification
Carbon Monoxide	99.99% min.
Oxygen	≤ 0.5 PPM
Nitrogen	≤ 10 PPM
Carbon Dioxide	≤ 20 PPM
Methane	≤ 5 PPM
Ethane	≤ 1 PPM
Propane	≤ 1 PPM
Dimethyl Ether	≤ 1 PPM
Hydrogen	≤ 1 PPM
Moisture	≤ 1 PPM

4. Properties:

Relative molecule mass	28.01
Critical point	–140.2 °C at 34.5 atm (3.5 MPa)
Melting point	–205.1 °C
Boiling point	–191.5 °C
Density, at 0 °C, 1 atm	1.250 g/litre
at 25 °C, 1 atm	1.145 g/litre
Specific gravity relative to air	0.967
Solubility in water at 0 °C, 1atm	3.54 ml/100 ml
at 25 °C, 1 atm	2.14 ml/100 ml
at 37 °C, 1 atm	1.83 ml/100 ml[a]
Conversion factors:	
at 0 °C, 1 atm	1 mg/m^3 = 0.800 ppm[b]
	1 ppm = 1.250 mg/m^3
at 25 °C, 1 atm	1 mg/m^3 = 0.873 ppm
	1 ppm = 1.145 mg/m^3

[a] Value obtained by graphic or calculated interpolation (Altman et al., 1971).
[b] Parts per million by volume

5. Analyses: ASTM D1946, "Analysis of Reformed Gas by Gas Chromatography (GC) with Thermal Conductivity Detection (TCD)", may be utilized to measure the quantity of CO present in gas mixtures. A copy of the method is Attachment 6.

III. Self-limiting levels of use

Studies of modified atmospheres for packaging meat that contained both higher and lower levels of CO have established that the 0.4% used in the AT2001 system both has advantageous characteristics and avoids disadvantages seen with lower or higher levels. A CO level of 0.4% is sufficient to produce stable, cherry red color, (Sørheim, et al. (1997), and use of CO through retail display time may result in masked spoilage.

IV. Basis of GRAS determination.

Pactiv believes its use of CO is GRAS based on scientific procedures, 21 CFR Sec. 170.30(b), and convened a panel of experts qualified by scientific training and experience to evaluate the safety of food, food additives and food ingredients. The experts have reviewed and evaluated the publicly available information summarized in this GRAS Notice. Their testimonial

letters are attached as Attachments 7 through 10. The above discussion and citations to generally available accepted scientific data, information, methods and principles relied upon, together with the anticipated consumption levels for both CO and meat treated with CO, provide ample basis to conclude that the use of CO at 0.4% in a modified atmosphere for packaging fresh meats is both safe and generally recognized as such by qualified experts.

The panel consisted of the following experts, whose GRAS opinions and curricula vitae are attached as attachments 7 through 10.

1. Daren Cornforth, Ph.D.
   Professor
   Department of Nutrition and Food Sciences
   Utah State University
   750 N. 1200 E.
   Logan, Utah 84322-8700

Dr. Cornforth is a professor in Nutrition and Food Sciences at Utah State University, Logan, Utah, and received his Ph.D. in food science and human nutrition from Michigan State University. He has performed extensive research and published multiple articles on the subject of meat color.

2. Vasilios Frankos, Ph.D.
   Principal
   Environ Corp.
   4350 N. Fairfax Dr.

Suite 300
Arlington VA 22203

Dr. Frankos is a Principal at ENVIRON corporation, Arlington, Virginia, a scientific consulting firm, and has over 20 years of experience in the toxicological and pharmacological evaluation of data used to assess the risks posed by foods, food additives, and other substances. He holds a Ph. D. from the University of Maryland Pharmacy School in Pharmacology and Toxicology.

3. Melvin C. Hunt, Ph.D
Professor
Weber Hall
Dept. of Animal Sciences and Industry
Kansas State University
Manhattan, KS 65506

Dr. Hunt is a professor of food science at the Department of Animal Sciences and Industry at Kansas State University, Manhattan, Kansas. He received his Ph.D. in food science at the University of Missouri. Among his many research projects and publications are multiple studies relating to meat color and the effects of various environments on meat color.

4. Oddvin Sørheim, Ph.D.
Senior Research Technologist
MATFORSK – Norwegian Food
Research Institute
Osloveien 1

N-1430 Ås
Norway

Dr. Sørheim is a Senior Research Technologist at the Norwegian Food Research Institute, Osloveien, Norway. He received his Ph.D. in food science from the Agricultural University of Norway, and has performed extensive research and industry consultation, and published numerous articles on meat, including extensive experience with the use of CO in modified atmosphere packaging of meat.

Pactiv is not aware of any reports of investigations that are inconsistent with the GRAS determination relating to the use described.

Conclusion

Based on all the above information, Pactiv Corporation has concluded that its use of 0.4% CO within the AT2001 modified atmosphere system for packaging fresh meat is Generally Recognized as Safe within the meaning of 21 U.S.C. Sec. 321(s).

Sincerely,

Eric F. Greenberg

Attachments:

Attachment 1	Authorization letter from Pactiv Corporation for representation by Eric F. Greenberg
Attachment 2	Photographs of meats treated with ActiveTech™ and ActiveTech™ 2001
Attachment 3	"Application for Assessment of the Food Additive Carbon Monoxide (CO) Prior to its Authorization", Norwegian Meat Cooperative; Norwegian Independent Meat Association (1999)
Attachment 4	"Evaluation of Beef Steaks and Ground Beef in the Pactiv ActiveTech™ Packaging System: Effects of Carbon Monoxide in the Package Atmosphere", Hachmeister, K; Hunt, M.; Milliken, G; May 2001.
Attachment 5	Certificate of Conformance, Carbon monoxide, Haun Welding Supply, Inc., Syracuse, New York, May 8, 2001.
..... Attachment 6	"Standard Practice for Analysis of Reformed Gas by Gas Chromatography", ASTM D 1946-90 (Reapproved 2000).
Attachment 7	Daren Cornforth, Ph.D., letter and curriculum vitae
Attachment 8	Vasilios H. Frankos, Ph.D., letter and curriculum vitae
Attachment 9	Melvin C. Hunt, Ph.D., letter and curriculum vitae
Attachment 10	Oddvin Sørheim, Ph.D., letter and curriculum vitae

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List of Attachments

Attachments:

ATTACHMENT 1

PACTIV
Advanced Packaging Solutions

August 9, 2001

Pactiv Corporation
Technology Center
2651 Brickyard Road
Canandaigua, New York 14424-1026

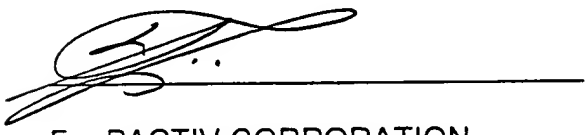
Division of GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St, SW
Washington, DC 20204

Re: Authorization of counsel regarding
NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT

To the FDA:

Please take note that Pactiv Corporation, with headquarters at 1900 West Field Court, Lake Forest, Illinois, 60045, authorizes its attorney, Eric F. Greenberg, 3500 Three First National Plaza, Chicago, Illinois 60602, to represent it and communicate on its behalf in all matters regarding Pactiv's NOTIFICATION OF CLAIM FOR GENERAL RECOGNITION OF SAFETY OF CARBON MONOXIDE IN A MODIFIED ATMOSPHERE SYSTEM FOR PACKAGING FRESH MEAT.

Sincerely,



For PACTIV CORPORATION

By: Vinod K. Luthra
General Manager
New Business Development
2651 Brickyard Road
Canandaigua, New York 14424

Summary regarding Pactiv Corporation

Pactiv Corporation, 1900 West Field Court, Lake Forest, Illinois, is a leading provider of advanced packaging solutions to customers around the world. The company employs 17,000 people in 87 facilities worldwide. Annual revenues exceed $3 billion.

Pactiv manufactures, markets and sells plastic and paper-based consumer products and food/foodservice packaging as well as protective and flexible packaging. Approximately 80% of its revenue comes from products made from different types of plastics, with the balance from paper and aluminum products.

The company's products include a wide range of items for consumers, food processors, supermarkets, foodservice entities, and the construction, automotive, computer, electronic, furniture and durable goods industries. The consumer products are sold under such recognized brand names as Hefty® , Baggies® , Hefty One-Zip® , Kordite™ and E-Z Foil®.

Pactiv further fuels internal growth by developing and commercializing proprietary new products and by designing value-added product-line extensions. In 1998, the consumer products and food/foodservice packaging business introduced over 80 new products and product-line extensions. In the protective and flexible packaging business, where custom design services drive revenues, Pactiv developed over 500 custom product applications in 1998. New product innovations include ActiveTech™ packaging, a proprietary modified atmospheric package used by food processors for case-ready meat.

ATTACHMENT 2

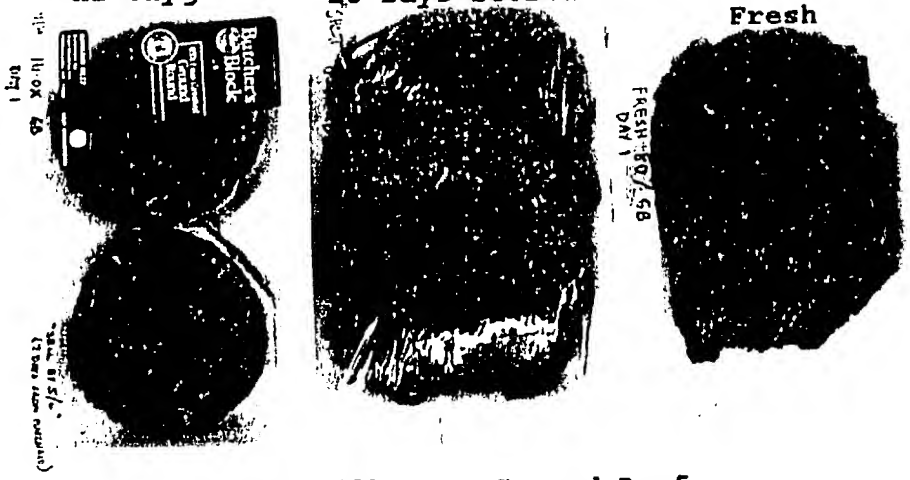
ActiveTech™ *Case Ready Packaging System*

For Case Ready Applications

000055

First Display Day

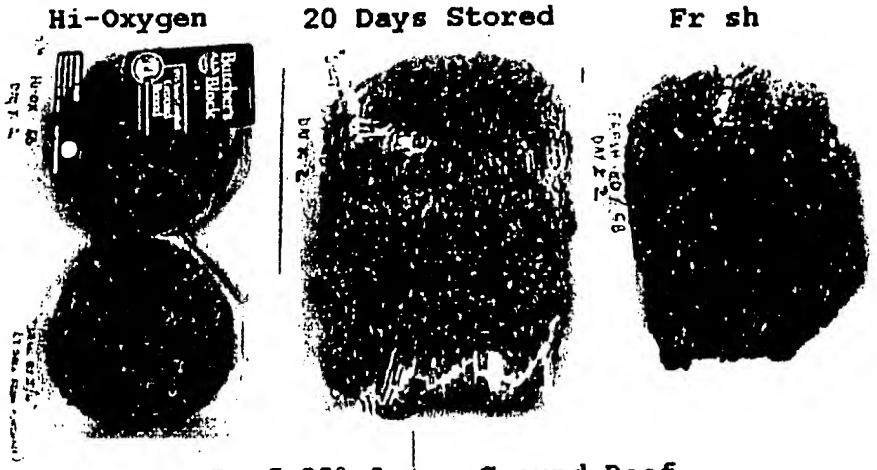
Hi-Oxygen | 20 Days Stored | Fresh



Beef: 80% Lean Ground Beef
Hi-OX, 20 Days Stored in "Short Gas", and Fresh
(0.4% CO/ 35% CO2/ 64.6% N2)

Second Display Day

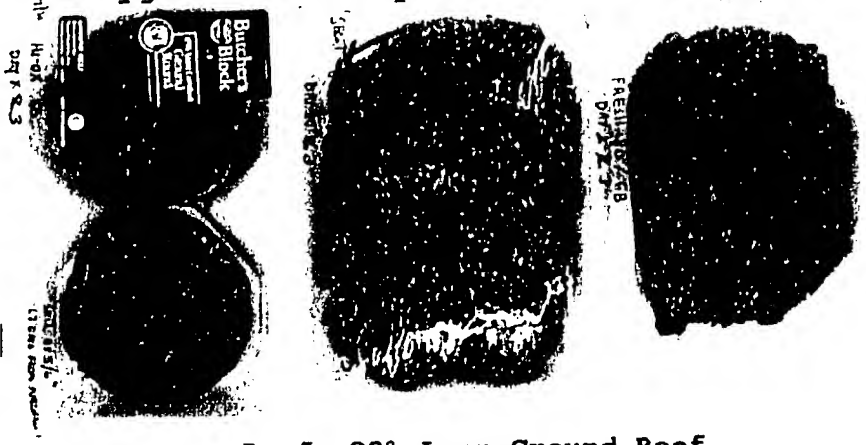
Hi-Oxygen | 20 Days Stored | Fr sh



Beef:80% Lean Ground Beef
Hi-Ox, 20 Days Stored in "Short Gas", and Fresh
(0.4% CO/ 35% CO2/ 64.6% N2)

Third Display Day

Hi-Oxygen | 20 Days Stored | Fresh



Beef: 80% Lean Ground Beef
Hi-Ox, 20 Days Stored in "Short Gas", and Freah
(0.4% CO/ 35% CO2/ 64.6% N2)

Fourth Display Day

Hi-Oxygen | 20 Days Stored | Fresh

Beef: 80% Lean Ground Beef
Hi-Ox, 20 Days Stored in "Short Gas", and Fresh
(0.4% CO/ 35% CO2/ 64.6% N2)

First Display Day

20 Days Stored

Fresh Cut

Beef: Top Round Steak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

Second Display Day

Fresh Cut

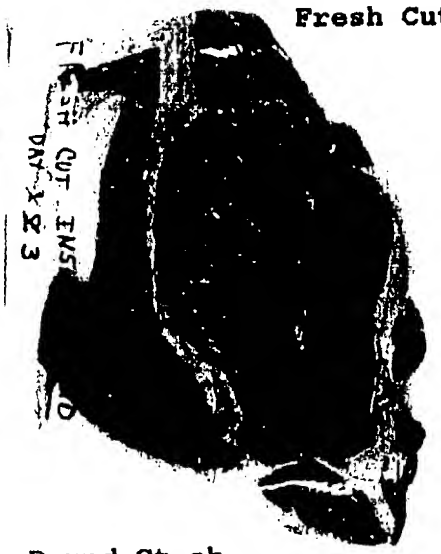
20 Days Stored

Beef: Top Round Steak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

Third Display Day

20 Days Stored

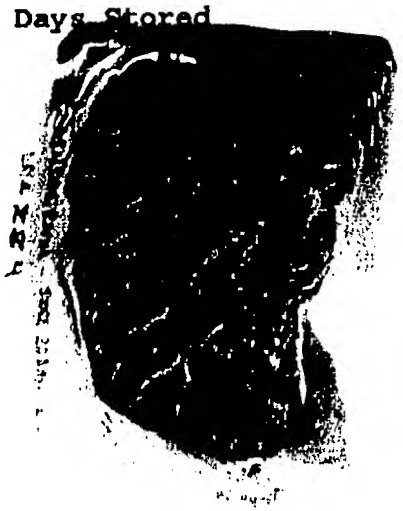
Fresh Cut



Beef: Top Round St ak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

Fourth Display Day

20 Days Stored



Fresh Cut

Beef: Top Round Steak
Fresh Cut and 20 Days Stored in "Short Gas"
(0.4% CO/ 35% CO2/ 64.6% N2)

0000057

The pages immediately following illustrate:

1. On the top of the photograph on the first page, an example is shown of the structure utilized for both ActiveTech™ and AT2001 incorporating tray, flexible overwrap, outer bag and activated oxygen scavenging sachet.

2. The second page of photographs show examples of ground meat color during the first, second, third and fourth days of display after packaging in (1) Hi-oxygen; (2) AT2001 atmosphere (referred to in captions as "Short Gas", 0.4% CO/35% CO2/64.6 N2), and after being held in that atmosphere for 20 days; and (3) Fresh.

3. The third page of photographs show examples of whole muscle meat (top round steak) color during the first, second, third and fourth days of display after packaging in (1) AT2001 atmosphere (referred to in captions as "Short Gas", 0.4% CO/35% CO2/64.6 N2), and after being held in that atmosphere for 20 days; and (2) Fresh cut.

ATTACHMENT 3

APPLICATION FOR ASSESSMENT OF THE FOOD ADDITIVE CARBON MONOXIDE (CO) PRIOR TO ITS AUTHORIZATION

(This application is based on the document "Presentation of an application for assessment of a food additive prior to its authorisation", Office for Official Publications of the European Communities, Luxembourg, 1989, ISBN 92-826-0135-8).

PART I. ADMINISTRATIVE DATA

I.1. Applicants: Altogether, the two applicants represent the total meat industry in Norway

Applicant no. 1:

The name of the applicant:

Norsk Kjøttsamvirke (Norwegian Meat Cooperative)

Address:

Lørenveien 37
P.O.Box 360 Økern
0513 Oslo, Norway

Other means of communication:

Telephone: +47 22 09 21 00
Fax: +47 22 15 59 08

Applicant no. 2:

The name of the applicant:

Kjøttbransjens Landsforbund (The Norwegian Independent Meat Association) – represents the private meat industry in Norway

Address:

Karoline Kristiansensvei 2, Fyrstikktorget
P.O.Box 6279 Etterstad
0603 Oslo, Norway

Other means of communication:

Telephone: +47 23 24 44 70, Fax: +47 23 24 44 80

I.2. The name of the manufacturer(s) of the substance:

RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy

I.3. The name of the person responsible of the dossier:

Research director Truls Nesbakken, Norwegian Meat Research Centre, P. O. Box 396 Økern, 0513 Oslo, Norway. Telephone +47 22 09 23 99, Mobile phone +47 91 87 81 46, Fax +47 22 22 00 16, e-mail: truls.nesbakken@fagkjott.no

I.4. The table of contents of dossier

This dossier is sent through the Norwegian Food Control Authority (Statens næringsmiddeltilsyn). Together with this document follow as enclosures:

1) Nissen, H., Alvseike, O., Bredholt, S., Holck, A. and Nesbakken, T. (submitted) Packaging of ground beef in an atmosphere with high carbon dioxide and low carbon monoxide restrains growth of *Yersinia enterocolitica, Listeria monocytogenes* and *Escherichia coli* O157:H7. Int. J. Food Microbiol. As long as this work is not published, please handle this information with care.

2) Nissen, H., Alvseike, O., Bredholt, S., Holck, A. and Nesbakken, T. (1999) Packaging of ground beef in an atmosphere with low carbon monoxide and high carbon dioxide restrains growth of *Escherichia coli* O157:H7, *Listeria monocytogenes, Yersinia enterocolitica* and *Salmonella diarizonae*. In: Tuijtelaars, A.C.J., Samson, R.A., Rombouts, F.M., Notermans, S., (Eds.), Food Microbiology and food safety into the next millenium. Proceedings of the Seventeenth International Conference of the International Committee on Food Microbiology and Hygiene (ICFMH), 13-17 September 1999, Veldhoven, The Netherlands, pp. 285-286.

3) Solheim, R. (1996) Consumer purchase probability of beef and pork packaged in different atmospheres. Report, Matforsk, 10 pp.

4) Sørheim, O. (1996) Discoloration of meat as an indicator of leakages in packages containing a CO gas mixture. Report, Matforsk, 5 pp.

5) Sørheim, O., Aune, T. and Nesbakken, T. (1997a) Technological, hygienic and toxicological aspects of carbon monoxide used in modified-atmosphere packaging of meat. Trends in Food Sci. Technol. 8, 307 - 312

6) Sørheim, O., Nissen, H., and Nesbakken, T. (1999) The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. Meat Sci. 52, 157 - 164

7) Letter from the director of Swedish Meats (which is the organisation of the Swedish meat cooperative) supporting the Norwegian meat industry's application to the EU Commission

8) Letter from the director of Swedish Meat Trade Association (which is the organisation of the private meat industry in Sweden) supporting the Norwegian meat industry's application to the EU Commission

9) Letter from the director of the Danish Pig Producers and Slaughterhouses, Copenhagen, Denmark supporting the Norwegian meat industry's application to the EU Commission

10) Letter from the Spanish Meat Industry's Association supporting the Norwegian meat industry's application to the EU Commission

11) Letter from the Finnish Meat Research Institute supporting the Norwegian meat industry's application to the EU Commission

PART II. TECHNICAL DATA

II.1. Name of the substance

- names in the IUPAC nomenclature: carbon monoxide
- other names (usual name/trade name/synonyms: carbon oxide, carbon monoxide)
- abbreviations: CO
- CAS number (if this has been attributed): 630 – 08 – 0

II.2. Specification of the substance

- composition (%, m/v, mg/kg), e.g. in the case of heterogeneous products): 100%
- empirical and structural formula: CO
- molecular weight: 28.010 g/mole
- degree of purity (%): higher than 99.3%
- nature of known impurities/percentage of significant and main impurities: (< 0.7%):

Impurities:	Concentration:
Oxygen + Argon	< 2500 vpm
Water	< 20 vpm
THC (Total hydrocarbons)	< 500 vpm
Hydrogen + Nitrogen	< 3500 vpm
Carbon dioxide	< 500 vpm

- physical form (liquid, powder, etc.): gas

- solubility (e.g. aqueous, organic solvents, lipid):

solubility in water, 0°C, a CO partial pressure of 101.325 kPa = 3.537 cm^3/100 cm^3. Solubility in organic solvents and lipid: not relevant – see Part II.6 Exposure

- other data that the applicant believes may be useful to identify the substance (e.g. physico-chemical properties, analytical data on differences between batches):

Thermodynamic properties of carbon monoxide as ideal gas at 25°C:
Heat capacity, c_p: 29.142 J/(mol * °K)
Entropy, S: 197.543 J/(mol * °K)
Enthalpy: 8.669 kJ/mol

- information on the microbiological characteristics, in particular on the possible presence of pathogens and bacterial or mycotoxins: not relevant

II.3. Manufacturing process

- Information on the method of manufacture (i.e. the process by which the raw materials are converted to the finished product):

The CO-gas is bought from RIVOIRA S.P.A., Torino, Italy (see I.2.). Hydro Rjukan Næringspark; P.O.Box 43-44, N-3661 Rjukan, Norway, makes the two CO-gas mixtures which are used in the Norwegian meat industry. They are called "Pakkemix NC1" and "DNC 29.7 – 0.3"

1) Pakkemix NC1 = 1.0% CO + 99% N_2

The production:

a) evacuation of an empty cylinder to under 10 mbar
b) flushing with N_2, quality 5.0
c) repeat the evacuation of an empty cylinder to under 1 mbar
d) manometric filling with CO, quality 2.3
e) manometric filling with N_2 quality 5.0
f) every tenth cylinder is analysed with gas chromatograph (GC) and thermoconductivity detector (TCD)

The pressure of the cylinder is 200 bar.

2) DNC 29.7 – 0.3 = 0.3% CO + 29.7% N_2 + 70% CO_2

The production:

a) evacuation of an empty cylinder to under 10 mbar
b) flushing with N_2, quality 5.0
c) repeat the evacuation of an empty cylinder to under 1 mbar
d) manometric filling with CO, quality 2.3
e) manometric filling with N_2, quality 5.0
f) manometric filling with CO_2, quality 3.0
g) every tenth cylinder is analysed with gas chromatograph (GC) and thermoconductivity detector (TCD)

The pressure of the cylinder is 50 bar..

II.4. Methods of analysis

- analytical methods to describe the substance, evaluate its purity and measure its physico-chemical and microbiological characteristics:

Oxygen + Argon, and Hydrogen + Nitrogen: Gas chromatograph with thermoconductivity detector (TCD) – min. detect. limits 10 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

THC: Gas chromatograph with flame ionization detector (FID) – min. detect. limit 0.5 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

Carbon dioxide: Gas chromatograph with helium ionization detector (HID) – min. detect. limit 0.5 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

Water: Specific water analyzer – min. detect. limit 0.1 vpm (RIVOIRA S.P.A., Stabilimento Chivasso gas, Via cardinal Massaia 75L, I-10147 Torino, Italy)

- analytical methods for the determination of the additive and its degradation products (where relevant), in the foodstuff of which the substance is to form part:

The isotope, C^{14}, might be used for measuring CO before and after heat treatment (Watts et al., 1978). Spectrophotometry is used to measure carboxymyoglobin at 540 and 577 nm (native) or 425, 542 and 570 nm (denaturated) (El-Badawi et al., 1964; Cornforth, 1994).

II.5. Justification for the additive

- intended use and purpose:

Fresh red meat (mainly beef, pork and lamb, but also horse, goat, reindeer, game etc.) packaged in an atmosphere with 60 - 70% carbon dioxide (CO_2), 30 - 40% nitrogen (N_2) and < 0.5% carbon monoxide (CO) (high CO_2/low CO mixture).

Gas mixtures with low concentrations of CO and high concentrations of CO_2 provide a combination of a long microbiological shelf life and a stable bright red colour of meat (Sørheim et al., 1999).

- the quantity to be added to specific foods and the residues in food: < 0.5% CO.

- investigations on the efficacy of the substance for the intended effect at the level proposed:

The main function of low levels of CO in modified atmospheres (MAs) is to give a stable, cherry red colour of the meat through strong binding of CO to myoglobin and formation of carboxymyoglobin (El-Badawi, 1964). Although a substantial increase in the shelf life of meat can be obtained by using various MAs, it is often limited by discolouration due to oxidation of myoglobin to metmyoglobin. This discolouration can be prevented by including a small fraction of CO in the gas mixture. Carboxymyoglobin is more resistant to oxidation than oxymyoglobin, due to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule (Wolfe, 1980). CO in concentrations of 1 - 5% had the ability to increase metmyoglobin reduction, even in the presence of air (Lanier et al., 1978).

The high CO_2/low CO mixture and absence of O_2 provides a unique combination of a long microbiological shelf life (caused by the high CO_2 level) and a stable cherry red colour (caused by the low CO level). CO_2 inhibits growth of many microorganisms, but it has no effect *per se* on the colour of the meat (Renerre and Labadie, 1993). This gas is absorbed in meat and fat tissue at a ratio of approximately 1 litre gas per kg tissue (Gill, 1988). N_2 affects neither the microbiology nor the colour of the meat, but prevents collapse of the packages because it is not absorbed in the product. O_2 supports the growth of aerobic microorganisms, and removal of O_2 from the MA will therefore extend the microbiological shelf life. The shelf life of meat is considerably longer in the high CO_2/low CO mixture than in the commonly used atmosphere of high oxygen (O_2) with approximately 70% O_2 and 30% CO_2. Consumption of meat treated with the high CO_2/low CO mixture will result only in negligible levels of carboxyhaemoglobin in blood. It is highly improbable that CO from packaging of meat will present a toxic threat to the consumer (Sørheim et al., 1997a).

Shelf life in the high CO_2/low CO mixture in comparison with alternative packaging methods:

Ground beef, beef loin steaks and pork chops were packaged in MAs of 0.4% CO/ 60% CO_2/ 40% N_2 (high CO_2/low CO mixture) and 70% O_2/ 30% CO_2 (high O_2 mixture). In addition ground beef was packaged in clipped chub packs, beef loin steaks were vacuum packaged, and pork chops were packaged in an atmosphere of 60% CO_2/ 40% N_2 with each pack containing an

O_2 absorber. The packs were stored in the dark at 4°C or 8°C for up to 21 days. Meat in the high CO_2/low CO mixture had a stable bright red colour. The storage lives in this gas mixture at 4°C, as limited by off-odours, were 11, 14 and 21 days for ground beef, beef loin steaks and pork chops, respectively. The high O_2 mixture resulted in an initially bright red to red colour of the meat, but the colour was unstable and off-odours developed rapidly. The off-odours probably were caused by *Brochothrix thermosphacta*, which grew in all meat types, and in ground beef by pseudomonads also. Meat stored in chub packs, vacuum packs and 60% CO_2/ 40% N_2 with an O_2 absorber developed off-odours and microflora similar to those of meat in low CO/high CO_2 mixture with however less acceptable colours or appearances. These results show that a low CO/high CO_2 atmosphere is effective for preserving retail-ready meat (Sørheim et al., 1999).

Aspects of spoilage:

Consumers may evaluate the shelf life of packaged meat based on its colour. A possible negative aspect of using CO in modified atmosphere packaging (MAP) of retail meat is a concern that the consumer might misjudge the product, because the microbiological status may be masked by the stable cherry red carboxymyoglobin colour (Knopf, 1980). However, the consumer is able to detect spoilage by off-odour (Sørheim et al., 1999). This is in contrast to ready to eat products such as cooked, sliced vacuum or gas packaged meat, gas packaged vegetables and vacuum-packaged cheeses where the consumers often have to taste it before judging the product as unacceptable. As ready to eat products, they also represent a higher risk than fresh meat packed in the high CO_2/low CO mixture which is heat treated before consumption. In the current low concentrations, below 0.5%, CO *per se* seems to have no or only minor effects on bacteria and the shelf life of the meat. The combination of CO with high concentrations of CO_2, for example 60 - 70%, is necessary for microbiological control. MAP enables centralised packaging operations with quality control, but MAP alone is no guarantee for the shelf life of the product. Sufficient shelf life can only be obtained through a proper quality control of raw materials, production, packaging, chill chain and retail conditions (Sørheim et al., 1999).

Pathogens in the high CO_2/low CO mixture in comparison with alternative packaging methods:

Growth of the pathogens *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and strains of *Salmonella* was compared in ground beef packed in high CO_2/low CO mixture, high O_2 mixture and in chub packs. The ground beef was inoculated with rifampicin or nalidixic acid/streptomycin-resistant strains (final concentration 10^2-10^3 bacteria/g) and stored at 4°C and 10°C for up to 14 days. At 4°C the shelf life based on stable colour and reduced background flora was prolonged for the high CO_2/low CO mixture, compared to the two other packaging methods, but at 10°C the shelf life was < 8 days for all the packaging methods. Growth of *Y. enterocolitica* was nearly totally inhibited both at 4°C and 10°C in the high CO_2/low CO mixture, while the bacterial numbers in the samples packed in the high O_2 mixture increased from about $5x10^2$ bacteria/g at day 0 to about 10^4 at day 5 at 4°C and to 10^5 at 10°C. Growth in the chub packs was even higher. *L. monocytogenes* showed very little growth at 4°C in all treatments. At 10°C there was slow growth of *L. monocytogenes* from about $5x10^3$ bacteria/g to about 10^4 at day 5 in the high CO_2/low CO mixture, while the numbers in the high O_2 mixture and the chub packs were about 10 times higher. Growth of *E. coli* O157:H7 at 10°C in the ground beef was nearly totally inhibited in both the high CO_2/low CO mixture and the high O_2 mixture. Growth of *E. coli* O157:H7 in the chub packs was higher reaching 10^5 bacteria/g on day

5. The *Salmonella* strains (*S. Typhimurium, S. Dublin, S. Enteritidis* and *S. enterica* «61:k1, 5,(7))» in the ground meat stored at 10°C for 5 and 7 days, grew to a higher number in the high CO_2/low CO mixture than in the high O_2 mixture. The present study shows that the prolonged shelf life at 4°C did not increase growth of *Y. enterocolitica* and *L. monocytogenes* in ground beef stored in the high CO_2/low CO mixture, but the observed growth of strains of *Salmonella* in this mixture and in chub packs at the abuse temperature of 10°C does emphasise the importance of temperature control during storage (Nissen et al. 1999; Nissen et al., submitted).

Consumer purchase probability:

Ground beef, beef loin steaks and pork chops were packaged in high CO_2/low CO mixture and high O_2 mixture. In addition ground beef was packaged in clipped chub packs, beef loin steaks were vacuum packaged, and pork chops were packaged in an atmosphere of 60% CO_2/ 40% N_2 with each pack containing an O_2 absorber. The purchase probability data were collected by interviewing 126 consumers usually purchasing meat and meat products. The consumers visually compared the samples within each type of meat after 4 days storage at 4°C. The consumers preferred ground beef packaged in the high CO_2/low CO mixture or the high O_2 mixture compared to ground beef packaged in clipped chub packs. Purchase probability increased when pork chops were packaged in the high CO_2/low CO mixture. Pork chops in packs containing an O_2 absorber, were rated lowest in purchase probabilities. The purchase probability for beef loin steaks was similar when packaged in the high CO2/low CO mixture or the high O2 mixture, and these products were preferred compared to beef loin steaks packaged in vacuum (Solheim, 1996)

- documentation on the need for the additive:

Alternatives to the high CO_2/low CO mixture:

The most common MA for retail packaging of meat today contains O_2 at high concentrations combined with CO_2, approximately 70% O_2/ 30% CO_2. The shelf life of meat in high O_2 atmospheres in commercial practice, typically at temperatures of 6 - 8°C, is about 7 days, limited by both microbiological spoilage and discolouration. Meat stored in the high O_2 mixture is often spoiled by bacteria like *Brochothrix thermosphacta* and pseudomonads (Gill, 1996). In MAs with high concentrations of O_2, the meat normally maintains its bright red oxymyoglobin colour for 4 - 7 days before the colour starts deteriorating into grey/brown due to formation of metmyoglobin (Sørheim et al., 1999). This length of time is often not considered sufficient for displaying and selling the product to 4.5 mill. inhabitants all along the distance from Kristiansand in the south to Kirkenes in the north of Norway (about 2700 km) corresponding to the distance from Oslo to Rome (about 2600 km)!

Using high CO_2, MAs of either CO_2 alone or CO_2/N_2 with up to 70% CO_2 increases the microbiological shelf life of the meat compared to MAs of high O_2. The absence of O_2 combined with the presence of CO_2 retard the microbiological growth. Unfortunately, the colour of the meat in MAs of CO_2 is less satisfactory, either as purple deoxymyoglobin or as grey/brown metmyoglobin. The meat inevitably discolours when concentrations of O_2 are low. Tolerance levels for avoiding metmyoglobin formation are less than 0.1% O_2 for beef (Gill and McGinnis, 1995) and 0.5% O_2 for pork (Sørheim et al., 1997b). These low O_2 levels, particularly for beef, are difficult to achieve in most commercial packaging operations, because small fractions of air will be

incorporated in the MAs of the packages. MAs with high CO_2 concentrations seem to be useful for retail packaging when combined with low concentrations of CO for stabilisation of myoglobin and the meat colour.

Vacuum is a packaging method commonly used for bulk storage, transportation and export of meat. However, vacuum has not been a successful method for retail packaging of meat, because of the purple deoxymyoglobin colour of the meat and the visible exudate that occurs in the packages (Bruce, 1990; Gill, 1996). Meat packaged in vacuum can not be presented in the bright red oxymyoglobin state, which depends on the presence of high concentrations of O_2 (Gill, 1996; Taylor et al., 1990), or alternatively as cherry red carboxymyoglobin with CO included in the MA.

Hazard for workers:

One of the objections raised against CO as a component of a packaging gas is the potential hazard it might represent for the workers in the meat plants. Using pure CO for mixing in the plant would certainly be such a risk, however, CO is delivered as a premixture (DNC 29.7 – 0.3) or in a 1% mixture together with 99% N_2 (Pakkemix NC1), which is the practice of gas suppliers to the Norwegian meat industry. This way of supplying CO is recognised to be a very safe handling procedure by the health authorities. MAs with concentrations of 60 - 70% O_2 must be handled carefully, because they are explosive gas mixtures. Strict safety regulations apply to explosive gas mixtures, resulting in additional costs of equipment and packaging operations. The benefit of the CO mixture is that it carries no risk or handling costs due to risk of explosion.

Experience of the Norwegian meat industry:

Despite the long term knowledge of CO and its many positive properties as a component of MAs for meat, the CO mixture has not been adopted to any large extent by the global meat industry. In many countries, like the US and EU, CO is presently not permitted for use in MAP of meat (Cornforth, 1994; European Parliament and Council Directive, 1995). However, the Norwegian food control authority has derogated from the EU directive for a two years period. Accordingly, the Norwegian meat industry might use CO as a component of a packaging gas in concentrations up to 0.5% until October 1, 2000. The high CO_2/low CO mixture is the only MAP which provides a shelf life sufficient for displaying and selling fresh retail meat products in all parts of Norway. The Norwegian meat industry started to use the high CO_2/low CO mixture in packaging of fresh retail meat products in the mid-eighties. The market share of retail meat packaged in the high CO_2/low CO mixture in Norway is currently estimated at 50 - 60% (ground beef as high as 85%). The Norwegian food control authority has not registered outbreaks or a higher frequency of sporadic cases of food borne diseases linked to such products (The Scientific Committee, under The Norwegian Food Control Authority, 19.4.99).

Support from the European meat industry:

The meat industry in Sweden has also discovered the benefits and advantages of the high CO_2 /low CO mixture in packaging of fresh meat. Both Swedish Meats (which is the organisation of the Swedish meat cooperative) and the Swedish Meat Trade Association (which is the organisation of the private meat industry in Sweden) support the Norwegian meat industry's

application to the EU Commission (letters enclosed). Also, the Danish Pig Producers and Slaughterhouses, the Spanish Meat Industry and the Finnish Meat Research Institute support the application (letters enclosed).

- **benefit for the consumer:**

The high CO_2/low CO mixture is the only MAP which provides a shelf life sufficient for displaying and selling fresh retail meat products in a large geographical area like Norway.

Food safety and traceability:

The high CO_2/low CO mixture enables centralised packaging operations with quality control with less risk for cross-contamination than in local butcher shops or by supermarket back-store operations. The Norwegian meat industry already produces pork products traceable in integrated systems back to the farm and beef products traceable to the individual animal.

The ability of *Y. enterocolitica* to multiply at low temperature is of considerable concern to food producers, particularly in countries like Australia, Canada, Denmark, Germany, New Zealand, Norway and Sweden where *Y. enterocolitica* has surpassed *Shigella* and now rivals *Salmonella* and *Campylobacter* as a cause of acute bacterial gastroenteritis (Nesbakken, 2000). The growth of *Y. enterocolitica* was totally inhibited in ground beef packed in the high CO_2/low CO mixture both at 4°C and 10°C while it grew fairly well both in the high O_2 mixture and in the chub packs.

At the abusive storage temperature of 10°C *E. coli* O157:H7 in the chub packs grew about as fast as the background flora. However, growth was nearly totally inhibited in the high CO_2/low CO mixture and in the high O_2 mixture (Nissen et al., 1999; Nissen et al., submitted).

Quality:

Centralised pre-packaging of retail meat in the meat industry is cost-effective compared to on-site packaging in food stores. Self-service food stores and supermarkets often require to be supplied with pre-packaged meat. The long shelf life of meat packaged in the high CO_2/low CO mixture provides a possibility of a wider selection of fresh meat on display in the stores. If the Norwegian meat industry looses the possibility to use this mixture, food stores in rural and remote areas will have to be supplied by frozen meat, which has a low acceptability of the consumer.

The high CO_2/low CO mixture provides extended freshness: fresh meat will last for many days (often more than a week) in the consumer's home refrigerator, and the consumer might shop fresh meat once a week, and fresh meat is available 24 hrs a day 7 days a week in hypermarkets, supermarkets, discount stores, service stores; and the consumer might get quality premium brand fresh meat in her/his local discount store. The consumer will find a wider variety of fresh meat products than otherwise possible.

The consumers seem to prefer fresh meat products packaged in the high CO_2/low CO mixture or the high O_2 mixture compared to other packaging methods (Solheim 1996).

Leakages in packages containing high CO_2/low CO mixture might be detected by the consumer. The discoloration might be an indicator of leakages for ground beef packed in the high CO_2/low CO mixture (Sørheim, 1996).

Prices:

Industrialised handling with centralised packaging in MAP means lower consumer prices. Waste due to "sell by date" in the distribution chain in Norway (high CO_2/low CO mixture) is less than 1%, as compared to 2 – 3% in countries using the high O_2 mixture, according to interviews with operators/supermarket chains in UK, The Netherlands and Spain (Dag Hallan, Norwegian Meat Cooperative, personal communication).

II.6. Exposure

Carbon monoxide (CO) is a colourless, odourless and tasteless gas. It is produced by incomplete combustion of carbon-containing organic material. The production of CO from natural processes is quite significant. Nevertheless, CO from antropogenic activities is far more important concerning human health, since this formation takes place in heavily polluted areas.

Natural background levels of CO are 0.01 - 0.9 mg/m^3 (0.01 - 0.8 ppm). In urban areas, 8-h mean concentrations of CO are generally < 20 mg/m^3, but levels exceeding 60 mg/m^3 have been reported (WHO, 1979). Among tobacco smokers, CO from smoking is by far the dominating source of CO exposure (WHO, 1987).

According to WHO experts (WHO, 1979; WHO, 1987), the only way of exposure which is of relevance to human health, is via inhalation of CO gas. Upon absorption from the lungs into the blood, CO forms a strong coordination bond with the iron atom in haemoglobin forming carboxyhaemoglobin (HbCO). The affinity of haemoglobin for CO is roughly 240 times that of its affinity for oxygen. CO is absorbed through the lungs and the concentration of HbCO in the blood will depend on several factors, mainly the concentration of CO in inhalation air, the exposure time and the level of activity of the individual (pulmonary ventilation).

The Norwegian meat industry is using a gas mixture of 60 - 70% CO_2, 30 - 40% N_2 and 0.3 - 0.4% CO for the packaging of fresh retail meat of beef, pork and lamb. According to Watts et al. (1978) beef which is exposed to an atmosphere containing 1% CO for 3 days result in about 30% saturation of the meat myoglobin. When the meat was cooked (hotplate maintained at 195°C for up to 8 minutes), only 0,1 mg CO remained in the meat per kg resulting in a loss of CO about 85%.

Data are very scarce concerning comparison of CO exposure from air and consumption of CO-treated meat. According to Sørheim et al. (1997a) consumption of 250 g CO-treated meat (after cooking) yield a theoretical intake of maximum 0.025 mg CO, compared with inhalation of 15 mg CO per hour at the acceptance level suggested by Norwegian authorities (giving 1.5% HbCO, including endogenous formation). Even though the estimates are crude, the calculations show without doubt that intake of CO from meat consumption is negligible. Furthermore, absorption of CO from the gastrointestinal tract will be very much lower (if it happens at all), compared with absorption via the lungs.

II.7. Reaction and fate in food

The main function of low levels of CO in MAs is to give a stable, cherry red colour of the meat through strong binding of CO to myoglobin and formation of carboxymyoglobin (El-Badawi, 1964). Although a substantial increase in the shelf life of meat can be obtained by using various MAs, it is often limited by discolouration due to oxidation of myoglobin to metmyoglobin. This discolouration can be prevented by including a small fraction of CO in the gas mixture. Carboxymyoglobin is more resistant to oxidation than oxymyoglobin, due to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule (Wolfe, 1980). CO in concentrations of 1 - 5% had the ability to increase metmyoglobin reduction, even in the presence of air (Lanier et al., 1978).

PART III. TOXICOLOGICAL DATA

III. 1 - 4.

Health effects of carbon monoxide has been evaluated by two WHO expert groups (WHO, 1979; WHO, 1987). The health effects are associated with the degree of HbCO formation. According to the aforementioned expert groups, the most sensitive individuals should be protected from CO exposures leading to a HbCO level exceeding 2.5%. In healthy adults, no adverse health effects are described at concentrations resulting in HbCO levels < 5%.

A small amount of CO is formed naturally in the human body, from breakdown of haemoproteins. This production leads to a HbCO concentration of about 0.5%. The average HbCO concentration in non-smokers is 1.2 - 1.5%, while the level in smokers usually is 3 - 4%.

The WHO experts (WHO, 1987) recommended a maximum HbCO level of 2.5 - 3% in order to protect the population at large, included sensitive individuals. In order to obtain this, they recommended maximum levels of CO in ambient air which will meet this requirement for different times of exposure:

Maximum levels of 100 mg/m^3 for < 15 min
Average levels < 60 mg/m^3 for 30 min
Average levels < 30 mg/m^3 for 1 hour
Average levels < 10 mg/m^3 for 8 hours

The European Union has not evaluated CO for use as a packaging gas for meat. However, in 1990 (European Commission, 1991), several other gases (carbon dioxide, oxygen, nitrogen, nitrous oxide, hydrogen and argon) were evaluated by the Scientific Committee for Food (SCF) for use as packaging gases and propellants. In this case it was considered unnecessary to adopt ADIs because of general knowledge of their safety in use, and the estimated insignificant intakes compared with exposure from other sources. Furthermore, in 1996, the SCF reviewed the safety of modified and controlled atmosphere packaging again, and placed particular emphasis on the importance of HACCP for the avoidance of microbiological risk in this context (European Commission, 1996). The SCF concluded that it does not see specific hazards for human health by

the use of controlled or MAs, but that a prerequisite is that the principles of HACCP are observed. A similar approach should also be feasible concerning CO used at very low concentrations in mixture with CO_2 and N_2.

Accordingly, the issue which should be solved concerning health effects of CO used in gas packaging, is the question of preventing the consumers from exposure to meat of unacceptable microbiological quality. Thus, two studies on shelf life, off-odour and colour (Sørheim et al., 1999) and pathogens (Nissen et al., 1999; Nissen et al., submitted) follow as enclosures. Summaries of the two studies are also given in "Part II.5. Justification for the additive - investigations on the efficacy of the substance for the intended effect at the level proposed".

III.5. Review of results and conclusions

As can be seen from the foregoing, exposure to CO via consumption of meat products treated with a MA containing < 0.5% CO represent a negligible source of CO, and will probably not contribute to any increase in the carboxyhaemoglobin level. From a toxicological point of view, packaging gas with < 0.5% CO presents no threat to human health (Sørheim et al. 1997a). This is in accordance with an assessment performed by members of The Scientific Committee for Food, under The Norwegian Food Control Authority (30.11.98).

PART IV. SUMMARY DOCUMENT

Gas mixtures with low concentrations of CO and high concentrations of CO_2 provide a combination of a long microbiological shelf life and a stable bright red colour of meat. Meat packaged in a MA with high O_2 can achieve an initial bright red colour, but the microbiological shelf life and the colour stability are considerably lower than those of the CO mixture. Using CO eliminates the need for having O_2 as a component of the MA. Other MAs and packaging methods, like high CO_2 with O_2 absorbers, chub packs and vacuum packs may give a microbiological shelf life similar to that of the high CO_2/low CO mixture, but with a less acceptable colour or appearance of the meat. Thus, there appears at present to be no fully satisfactory alternatives to the CO mixture used in packaging of retail-ready red meats in Norway (Sørheim et al., 1999).

In an investigation, growth of *E. coli* O157:H7 at 10°C in ground beef was nearly totally inhibited in the high CO_2/low CO mixture. The prolonged shelf life at 4°C did not increase growth of *L. monocytogenes* in ground beef stored in the high CO_2/low CO mixture. The growth of *Y. enterocolitica* was totally inhibited in ground beef packed in the high CO_2/low CO mixture both at 4°C and 10°C while it grew fairly well both in the high O_2 mixture and in the chub packs. However, the observed growth of strains of *Salmonella* both in the high CO_2/low CO mixture and in chub packs at the abuse temperature of 10°C does emphasise the importance of temperature control during storage (Nissen et al., 1999; Nissen et al., submitted).

From a toxicological point of view, packaging gas with < 0.5% CO presents no threat to human health (Sørheim et al. 1997a). The European Union has not evaluated CO for use as a packaging gas for meat. However, in 1990 (European Commission, 1991), several other gases (carbon dioxide, oxygen, nitrogen, nitrous oxide, hydrogen and argon) were evaluated by the Scientific

Committee for Food (SCF) for use as packaging gases and propellants. In this case it was considered unnecessary to adopt ADIs because of general knowledge of their safety in use, and the estimated insignificant intakes compared with exposure from other sources. A similar approach should also be feasible concerning CO used at very low concentrations in mixture with CO_2 and N_2.

The Norwegian meat industry started to use the high CO_2/low CO mixture in packaging of fresh retail meat products in the mid-eighties. The market share of retail meat packaged in the high CO_2/low CO mixture in Norway is currently estimated at 50 - 60% (ground beef as high as 85%). The Norwegian food control authority has not registered outbreaks or a higher frequency of sporadic cases of food borne diseases linked to such products (The Scientific Committee, under The Norwegian Food Control Authority, 19.4.99).

Conclusions:

Gas mixtures with low concentrations of CO, up to 0.5%, and high levels of CO_2, approximately 70%, have many advantages regarding shelf life, inhibition of pathogenic bacteria like *E. coli* O157 and *Y. enterocolitica*, colour stability, labour safety and costs. CO used as described in these concentrations, does not present any toxic threat to the consumer. Considering the benefits the Norwegian meat industry has experienced with the CO gas mixture over the past decade, this gas mixture should have a potential for a wider application in retail packaging of meat in the EU.

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PACKAGING OF GROUND BEEF IN AN ATMOSPHERE WITH HIGH CARBON DIOXIDE AND LOW CARBON MONOXIDE RESTRAINS GROWTH OF *YERSINIA ENTEROCOLITICA, LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI* O157:H7

H. Nissen*, O. Alvseike[1,2], S. Bredholt, A. Holck and T. Nesbakken[2]

MATFORSK, Norwegian Food Research Institute, Oslovn. 1, N-1430 Ås, Norway

[1]The Norwegian School of Veterinary Science, P.O. Box 8146 Dep., 0033 Oslo, Norway

[2]Norwegian Meat Research Centre, P.O. Box 396, 0513 Oslo, Norway

Corresponding author and request for reprints: Hilde Nissen, MATFORSK, Norwegian Food Research Institute, Oslovn. 1, N-1430 Ås, Norway

Tel: +47 64970100

Fax: +47 64970333

E-mail: hilde.nissen@matforsk.no

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Abstract

Growth of the pathogens *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and strains of Salmonella was compared in ground beef packed in modified atmospheres of 60 % CO_2/ 40 % N_2 /0.4 % CO (high CO_2/ low CO mixture), 70 % O_2/ 30 % CO_2 (high O_2 mixture) and in chub packs. The ground beef was inoculated with rifampicin-resistant or nalidixic acid/streptomycin-resistant strains (final concentration 10^2-10^3 bacteria/g) and stored at 4 and 10 °C for up to 14 days. At 4 °C the shelf life based on stable colour and reduced background flora was prolonged for the high CO_2/ low CO mixture compared to the two other packaging methods, but at 10 °C the shelf life was < 8 days for all the packaging methods. Growth of *Y. enterocolitica* was nearly totally inhibited both at 4 and 10 °C in the high CO_2/ low CO mixture, while the bacterial numbers in the samples packed in the high O_2 mixture increased from about $5x10^2$ bacteria/g at day 0 to about 10^4 at day 5 at 4°C and to 10^5 at 10°C. Growth in the chub packs was even higher. *Listeria monocytogenes* showed very little growth at 4 °C in all treatments. At 10 °C there was slow growth from about $5x10^3$ bacteria/g to about 10^4 at day 5 in the high CO_2/ low CO mixture, while the numbers in the high O_2 mixture and the chub packs were about 10 times higher. Growth of *E. coli* O157:H7 at 10 °C in the ground beef was nearly totally inhibited in both the high CO_2/ low CO mixture and the high O_2 mixture. Growth in the chub packs was higher, reaching 10^5 bacteria/g on day 5. The *Salmonella* strains (*S.* Typhimurium, *S.* Dublin, *S.* Enteritidis and *S. enterica* 61:k:1,5,(7)) in the ground meat stored at 10 °C for 5 and 7 days grew to a higher number in the high CO_2/ low CO mixture than in the high O_2 mixture. This study shows that the prolonged shelf life at 4 °C did not increase growth of *Y. enterocolitica*

and *L. mononcytogenes* in ground beef stored in the high CO_2/ low CO mixture mixture, but the observed growth of strains of salmonella at 10 °C in this mixture and in chub packs does emphasise the importance of temperature control during storage.

Keywords:

Ground beef, modified atmosphere packaging, high CO_2, carbon monoxide, *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7.

1. Introduction

Ground beef for retail sale is most often ready-packed in modified atmospheres (MA) or in chub packs. MA-packed ground beef has a longer microbiological shelf life and also maintains an attractive red colour. For the past decade the Norwegian meat industry has been using a gas mixture of 60-70 % CO_2, 30-40 % N_2, 0.3-0.5 % CO. (The CO comes ready mixed in the N_2 from the supplier.) The reason for adding CO to the gas mixture is that it will produce a long-lasting cherry-red colour of the meat (Sørheim et al., 1999), but the low concentration of CO has little effect on the microflora of the meat (Clark et al., 1976; Gee and Brown, 1978; Luno et al., 1998). The use of CO at such low concentrations does not present any toxic threat to the consumers (Sørheim et al., 1997). The most commonly used gas mixture for retail-ready meat in other European countries is 70 % O_2/30 % CO_2 (Gill, 1996). The high oxygen concentration is needed to keep the red colour of the meat (Lambert et al., 1991). It is therefore only possible to obtain half the CO_2 concentration used in the high CO_2/ low CO mixture. The microbiological shelf life of the high O_2 mixture will be longer than in air, but less than in the high CO_2/ low CO gas mixture (Sørheim et al., 1999).

The inclusion of CO is controversial because the stable cherry–red colour can last beyond the microbiological shelf life of the meat and thus mask spoilage (Kropf, 1980). The extended shelf life obtained by MAP may under some conditions imply increased risk of growth of pathogens (Silliker and Wolfe, 1980; Hintlian and Hotchkiss, 1986; Farber, 1991; Lamberts et al., 1991). This issue has also been discussed by the European Commission (1997).

However, even if meat packed in high CO_2/ low CO mixture acquires a stable colour, the shelf life based on odour is significantly longer in the high CO_2/ low CO

mixture only at 4 °C (Sørheim et al., 1999). At this temperature *Yersinia enterocolitica* and *Listeria monocytogenes* are considered to be the most serious pathogens in meat. At abuse temperatures (>8 °C) *Escherichia coli* O157:H7 and *Salmonella* spp. also may grow and increase the health risk to the consumers. In the present study we wanted to compare growth of these pathogens in ground beef packed in a commercial Norwegian 60 % CO_2/40 % N_2/0.4 % CO (high CO_2/low CO mixture) with growth in a high O_2 (70 % O_2/30 % CO_2) gas mixture and in ground beef in chub packs during storage at 4 and 10 °C in order to evaluate the microbiological safety of the product.

2. Materials and methods

2.1. Preparation and packaging of the ground beef

The beef carcasses were de-boned, and trimmings with 14 % fat were ground through a 4 mm plate. The batch of ground beef was divided into 500 g portions which were packaged in 0.4 % CO/ 60 % CO_2/ 40 % N_2 (high CO_2/ low CO mixture), 70 % O_2/ 30 % CO_2 (high O_2) or packed in clipped chub packs. The beef was packed at a commercial meat plant within 1 hour of grinding as described by Sørheim et al. (1999).

2.2. Bacterial cultures and growth conditions

Strains of the following pathogens were inoculated in the ground beef: *Yersinia enterocolitica* (mixture of 3 strains), *Listeria monocytogenes* (mixture of 3 strains isolated from cooked sausage, Blom et al., 1997, Nissen and Holck, 1999), *Escherichia coli* O157:H7, NCTC 1200 (National Collection of Type Cultures,

Colindale, London), non-toxic strain (resistant to 100 µg/ml nalidixic acid and 1000 µg/ml streptomycin) and *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,(7) (*S. enterica* 61:k:1,5,(7)), mixture of 3 strains (National Institute of Public Health, Oslo). The listeria and yersinia strains were made resistant to rifampicin by spreading 0.1 ml of overnight cultures onto agar plates of TSB medium (Oxoid, CM 129) containing 50 µg/ml rifampicin (Sigma, St.Louis, MO, USA). The growth rates of the resistant strains were practically equal to those of the parent strains when tested in TSB medium in a Bioscreen instrument (Labsystem Co., Helsinki, Finland) at the same temperature, pH and a_w (NaCl) concentrations.

In a second experiment four rifampicin-resistant salmonella strains, *S.* Typhimurium, *S.* Dublin, *S.* Enteritidis and *S. enterica* 61:k:1,5,(7) were used to inoculate the MAP- packed ground beef. The growth rates (measured as above) of the resistant strains of *S.* Enteritidis and *S. enterica* 61:k:1,5,(7) were essentially the same as the parent strains while the growth rates of *S.* Dublin and *S.* Typhimurium were slightly lower.

2.3. Inoculation and storage

After packaging the ground beef was inoculated with stationary cultures (the bacteria were cultivated overnight at 30°C and kept in the refrigerator for 1 day before use) of the different pathogenic bacteria. The stock cultures were diluted in peptone water (PW) (Bacto peptone, Difco, 1g/l; NaCl, Merck, 8.5 g/l) and the strains belonging to the same species or serovars were mixed. Fifty µl of each pathogen were inoculated with a syringe through a gas probe self-sealing tape (Toray Engineering Co. Ltd, England) into one of the corners of the MA packages. The packages thus had one pathogen inoculated in each corner. In the chub packs the

pathogens were inoculated at least 3 cm apart. Packages inoculated only with *Y. enterocolitica* and *L. monocytogenes* only were stored at 4°C and analysed after 0, 2, 5, 8 and 14 days while packages inoculated with all 4 pathogens were stored at 10 °C and analysed after 0, 2, 5 and 8 days.

In the second experiment four serovars of «*Salmonella*» were inoculated in one corner each of the package of ground beef and which was stored at 10 °C and analysed after 0, 2, 5 and 7 days. Non-inoculated packages used as controls were also stored at 10 °C.

2.4. Microbial analyses

Samples of 25 g ground beef containing the inoculated pathogens were transferred to a stomacher bag and mixed with 150 ml peptone water (8.5 g NaCl, 1.0 g peptone/1000 ml water). One hundred µl of a ten-fold dilution series were plated on blood agar containing 50 µg/ml rifampicin for *L. monocytogenes* and *Y. enterocolitica* or 100 µg/ml nalidixic acid and 1000 µg/ml streptomycin sulphate for *E. coli* O157:H7. From the undiluted mixture an aliquot of 1 ml was also plated out. For enumeration of *Salmonella* spp. the selective medium Brilliant Green Agar (modified) (BGA; Oxoid, Basingstoke, Hampshire, England) was used. The colonies were confirmed on Triple Sugar Iron Agar (TSI; Difco, Detroit, MI,) and Urea agar (Urea Agar Base, Oxoid CM53 and Urea Solution, Oxoid SR20) followed by agglutination by monovalent antisera (provided by the National Institute of Public Health). In the second experiment, samples for detection of the four salmonella strains were plated on blood agar containing 50 µg/ml rifampicin samples from non-inoculated packages were treated the same way and plated on MRS plates (CM359, Oxoid), pH 5.7, for determination of lactic acid bacteria and PCA (Difco, Detroit, MI, USA) plates for total

counts of bacteria. The plates were incubated at 30°C for up to 2 days, all aerobically. On each sampling date the packs with MA were analysed for O_2 and CO_2 and the pH for all samples was measured in the stomacher solution. Samples from two replicate packages were used for all analyses, except after 7 days storage in experiment 2 where three replicate packages were analysed.

2.5. Statistical analyses

Microbial data were subjected to analysis of variance (ANOVA) and Tukey's pairvise comparisons. It was deemed appropriate to perform ANOVA on these data after a $\log_{10}$ transformation, thereby obtaining a distribution more akin to the normal distribution on which ANOVA is based.

3. Results

As expected the shelf life of the ground beef stored at 4 °C was prolonged in the high CO_2/ low CO mixture compared with the other packaging methods. This was due to the stable colour and reduced background flora resulting in little off-odour. Thus the ground beef packed in the high CO_2/ low CO mixture still had an acceptable smell after 14 days of storage at 4 °C, while the beef packed in high O_2 mixture and in the chub packs had some off-odours. The difference in shelf life was less at 10 °C. After 5 days storage the ground beef packed in the high CO_2/ low CO mixture had an acceptable smell (except the packages inoculated with salmonella, while beef packed in the high O_2 mixture and the chub packs had a slight off-odour.

After 8 days storage there was a strong off-odour for all treatments, but the ground beef in the high CO_2/ low CO mixture still looked bright red, in accordance with Sørheim et al. (1999). The O_2 content in the high CO_2/ low CO mixture was virtually

zero throughout storage at both temperatures. At 10 °C the O_2 content in the high O_2 gas mixture decreased from 70 to about 35 % after 8 days storage, probably due to aerobic bacterial metabolism. The chub packs had an O_2-permeable casing which probably was the cause of the high bacterial growth in these packs at both temperatures.

Growth of *Y. enterocolitica* was totally inhibited both at 4 and 10 °C in the high CO_2/ low CO mixture (Fig. 1a and b), while the number in the samples packed in the high O_2 mixture increased from about $5x10^2$ cfu/g at day 0 to about 10^4 cfu/g at day 5 at 4 °C and to 10^5 cfu/g at 10°C. Growth in the chub packs at 4 °C was even higher than in the other treatments. Growth in chub packs was also higher than in high O_2 at 10 °C p=0.007). *L. monocytogens* (Fig. 2a) showed very little growth at 4 °C in all treatments. At 10 °C (Fig. 2b) there was slow growth (from about $5x10^3$ bacteria/g to about 10^4 at day 5) in the high CO_2/ low CO mixture. This was more than 10-fold higher cfu/g at day 5 than in the high O_2 mixture (p= 0.040) and the chub packs (p=0.035). Ground beef inoculated with *E. coli* O157:H7 and strains of salmonella was stored at 10°C. Growth of *E. coli* O157:H7 was slow both in the high CO_2/ low CO mixture and the high O_2 mixture (Fig. 3) and the numbers were less than 10^4 cfu/g at day 5. Growth in the chub packs was greater than in the high CO_2/ low CO-mixture (p=0.011) and in the high O_2 mixture (p=0.019), reaching 10^5 cfu/g. Growth of lactic acid bacteria in the non-inoculated packages was somewhat inhibited in the high CO_2/ low CO mixture, especially at 4 °C (Fig. 4). At start of the experiment the pH in the ground beef was about 5.8 in all packages. After 5 days storage the pH was about 5.7 in the high CO_2/ low CO mixture, 5.5 in the high O_2 mixture and 5.3 in the chub packs.

Due to growth of other bacteria on the selective plates, only approximate numbers of *S. enterica* 61:k:1,5,(7) were obtained, but growth of about 1.5 log units was observed both in the CO mixture and the chub packs (results not shown). This increase was not seen in the high O_2 mixture. To verify these results and check whether they were valid for other serovars more virulent to humans, such as *S.* Typhimurium, *S.* Dublin and *S.* Enteritidis, a second experiment was performed. The results (Fig. 5 a, b, c and d) show that after 2 days of storage at 10 °C there was essentially no growth of the salmonella strains in ground beef packed in the high CO_2/ low CO mixture and in the high O_2 mixture, while the numbers of salmonella in the chub packs were about 10 fold higher. After 5 days there was a slight off-odour in all the packages except for one package with high CO_2/ low CO mixture which smelled strongly of H_2S. In this package the numbers of all the salmonella strains were higher than in the replicate package and were of the same magnitude as the numbers in the chub packs. In the O_2 mixture there was no growth of *S.* Dublin and *S.* Enteritidis and only a low growth of *S. enterica* 61:k:1,5,(7) and *S.* Typhimurium. The growth of the salmonella strains was still greatly inhibited in the high O_2 mixture, while growth in the high CO_2/ low CO mixture was just as high or even higher than in the chub packs.

In the non-inoculated packages the lactic acid bacteria rapidly constituted most of the background flora (not shown). After 5 days storage the numbers were higher in the chub-packed samples, but after 8 days there were no obvious differences (Fig. 6). The pH in the non-inoculated ground beef followed the same pattern as in experiment 1.

4. Discussion and Conclusions

Ground beef is a high-risk product because pathogens may be mixed into the ground product which may not be sufficiently heated before consumption. To inhibit growth of spoilage bacteria and increase shelf life, MAP is often used by retailers. The question «Do modified atmospheres enhance risk to the consumers health, but delay signs of spoilage» raised by Hintlian and Hotchkiss (1986) is therefore relevant. When evaluating the safety of ground beef in the high CO_2/ low CO mixture compared to other commercially available packaging methods, we have focused on bacteria that show good growth below 10 °C and are most relevant for meat products.

The ability of *Y. enterocolitica* to multiply at low temperature is of considerable concern to food producers, particularly in countries like Australia, Canada, Denmark, Germany, New Zealand, Norway and Sweden where *Y. enterocolitica* has surpassed *Shigella* and now rivals *Salmonella* and *Campylobacter* as a cause of acute bacterial gastroenteritis (Nesbakken, 1999). In our study, growth of *Yersinia enterocolitica* was totally inhibited in ground beef packed in the high CO_2/ low CO mixture even at 10 °C while it grew fairly well both in the high O_2 mixture and in the chub packs. Manui-Tawiah et al. (1993) found that pork shops packed in different MA with 20 or 40 % CO_2 with or without O_2 allowed growth of *Yersinia enterocolitica*, but here the CO_2 concentration was lower than in the high CO_2/ low CO mixture (60 %) used in our study.

Listeria monocytogenes is also a pathogen that grows well at low temperatures, but in our study there was no growth of this bacterium in the ground beef in any of the packages at 4 °C, and only slow growth at 10 °C. This agrees with results of

Farber and Daley (1994) who found no growth of *L. monocytogenes* in different meat products when stored at 4 °C.

At the abusive storage temperature of 10 °C, *E. coli* O157:H7 in the chub packs grew about as fast as the background flora. However, growth was nearly totally inhibited in the high CO_2/ low CO mixture and in the high O_2 mixture. This is in accordance with the predictive model of Sutherland et al. (1997). Their study showed that *E. coli* O157:H7 is relatively tolerant of CO_2, but growth could be inhibited at 10 °C at high CO_2 concentrations and pH < 6.0.

In our study, growth of *Salmonella* spp. was not inhibited in ground beef packed in high CO_2/ low CO mixture and stored at 10 °C, contrary to what is found in many other studies (e.g. D'Aoust, 1991). Although salmonella may grow well and out-compete the background flora on fresh meat stored at 10 °C (Alford and Palumbo, 1969; Mackey and Kerridge, 1988), most reports claim that growth will be inhibited in MAP at this temperature (Siliker and Wolfe, 1980; D'Aoust, 1991; Gill and DeLacy, 1991). Nychas and Tasson (1996) found that high CO_2 atmospheres were more inhibitory for growth of *S.* Enteritidis on fresh poultry at 10 °C than were high O_2 atmospheres, the opposite of what we found for ground beef. Inhibition of bacterial growth may, however, be influenced by pH, texture and the composition of the product, and Gill and DeLacy (1991) did find growth of *S.* Typhimurium in high-pH beef packed in CO_2 and stored at 10 °C. Oxidative stress reactions in salmonella have recently been reported (Stephen et al., 1999). This may explain the inhibition of growth (longer lag phase) in the high O_2 mixture in our study.

The present study shows that the prolonged shelf life (due to stable colour and reduced background flora) at 4 °C did not increase the risk of growth of *Y. enterocolitica* and *L. monocytogenes* in ground beef stored in the high CO_2/ low CO

gas mixture. This is probably due to the high CO_2 concentration that is inhibitory to most microorganisms (Dixon and Kell, 1989). Even at the abusive temperature of 10 °C, the numbers of pathogens at the end of the shelf life (5 days) were less or the same as were found in the chub packs. The observed growth of salmonella in the CO mixture and chub packs does however emphasise the importance of temperature control during storage. There is a wide range of temperature criteria for chilled foods at retail in European countries. The values range from –1 °C to 10 °C, with most temperatures being between 4 and 8 °C (European Commission, 1996). These aspects should also be considered together with the conclusions of the EU report (European Commission, 1997) which state that MAP has proven to enhance the product quality by inhibiting the spoilage bacteria. MAP may also constitute a hurdle to the growth of some pathogens, and the safety of MAP products are mostly threatened by temperature abuse.

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Fig. 1. Growth of *Yersinia enterocolitica* inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a**, 4 °C or **b**, 10 °C.

Fig. 2. Growth of *Listeria monocytogenes* inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a**, 4 °C or **b**, 10 °C.

Fig. 3. Growth of *Escherichia coli* O157: H7 inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs, stored at 10 °C.

Fig. 4. Growth of lactic acid bacteria (cfu/g on MRS, pH 5.7) in non-inoculated ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a**, 4°C or **b**, 10 °C.

Fig. 5. Growth of strains of *Salmonellae* inoculated in ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2 (70 % O_2/ 30 % CO_2) or in chub packs, stored 10 °C. **a.** *S.*Typhimurium **b.** *S.* Dublin **c.** *S.* Enteritidis **d.** *S. enterica* 61:k:1,5,(7).

Fig. 6. Growth of lactic acid bacteria (cfu/g on MRS, pH 5.7) in non-inoculated ground beef packed in high CO_2/ low CO mixture (0.4 % CO/ 60 % CO_2/ 40 % N_2), high O_2

(70 % O_2/ 30 % CO_2) or in chub packs. The ground beef was stored at **a,** 4 °C or **b,** 10 °C.

Fig. 1a

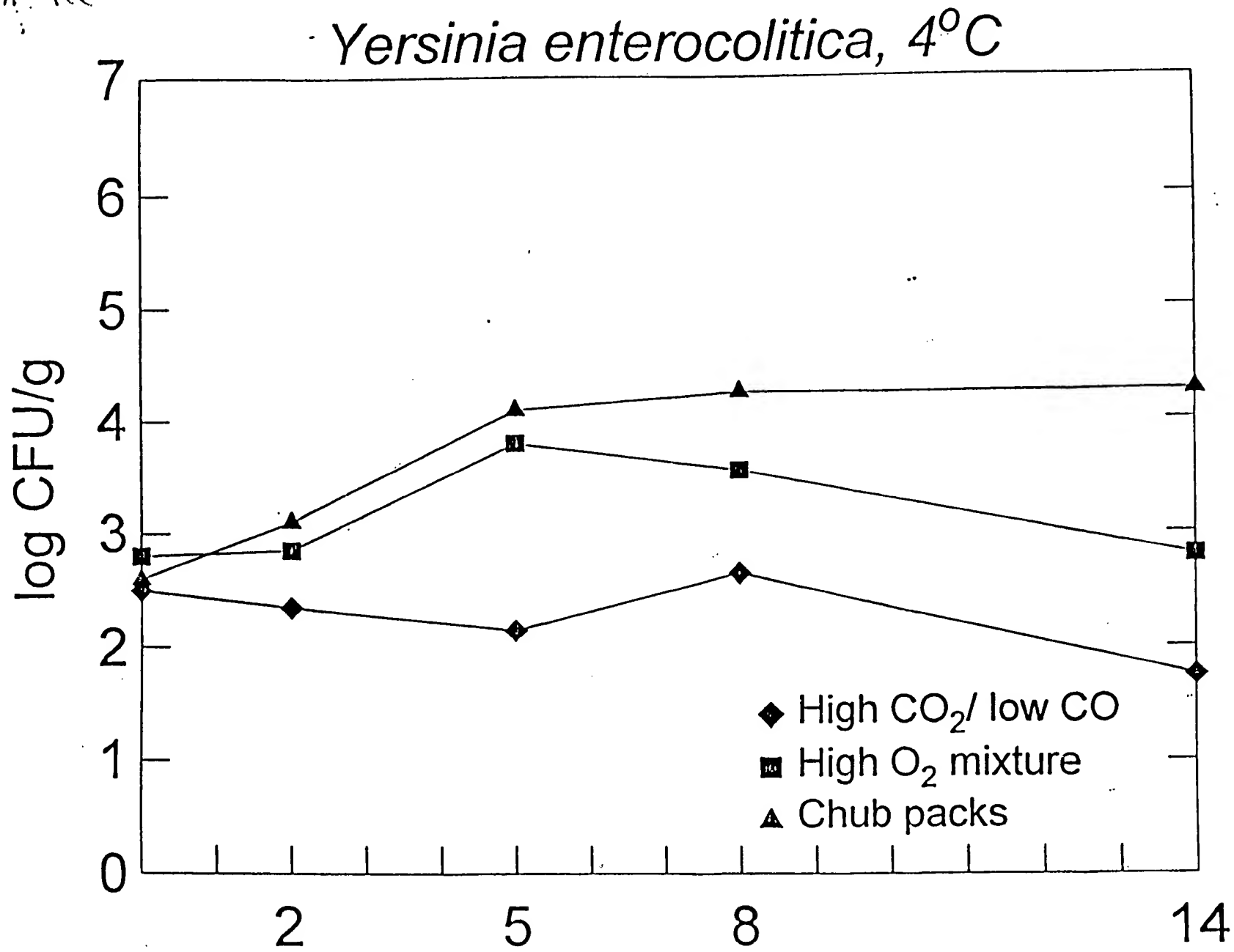

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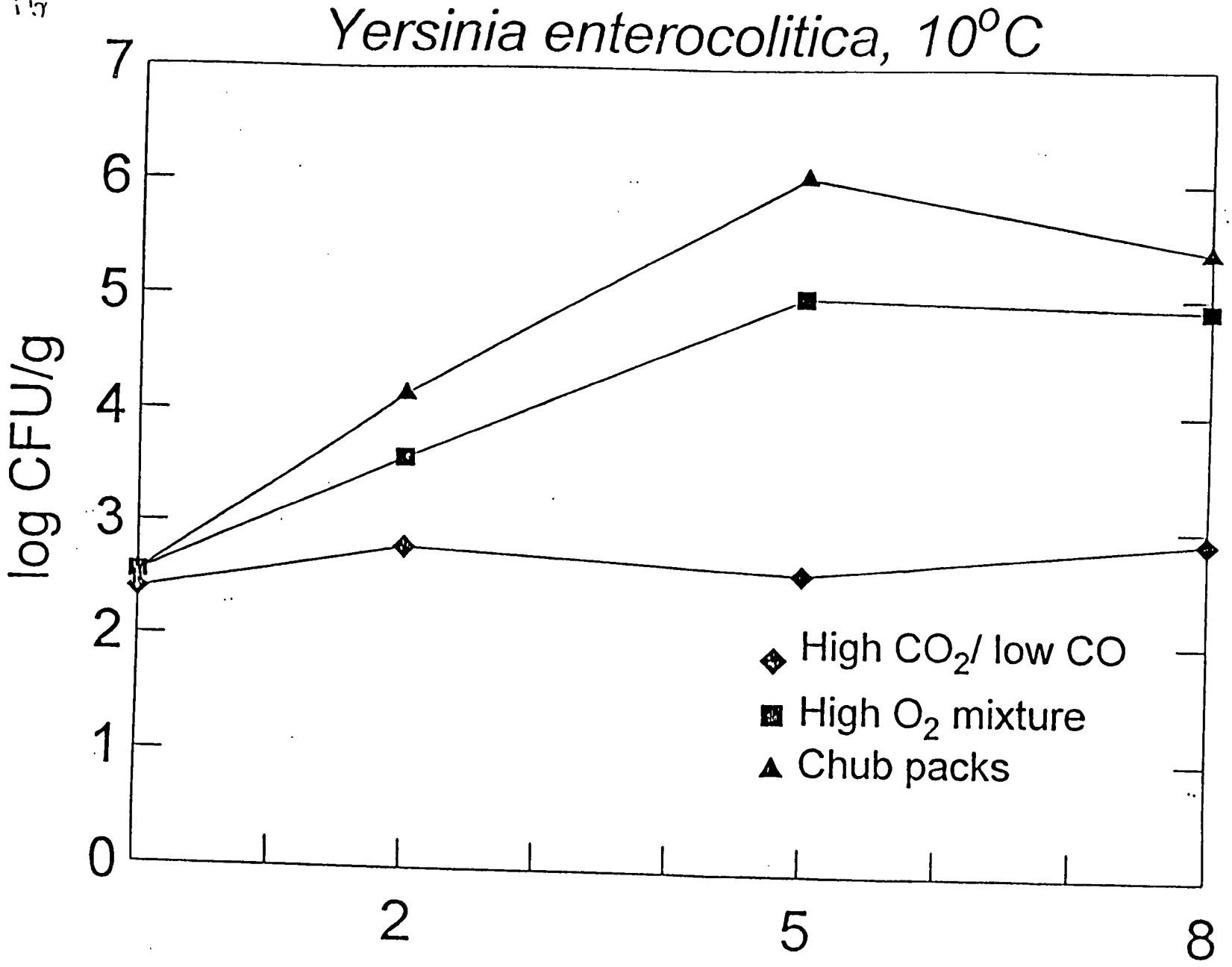
Fig 16



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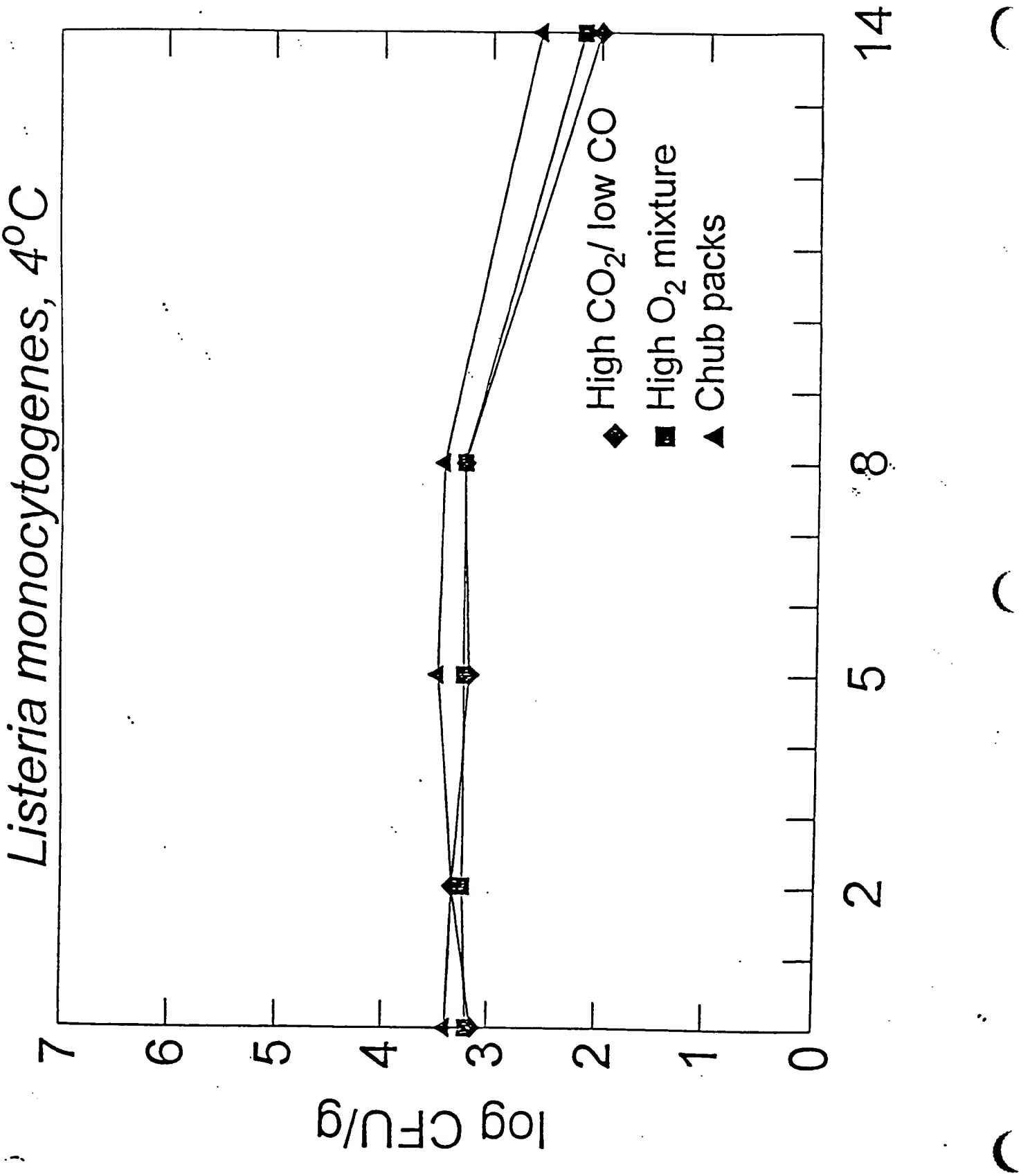
Listeria monocytogenes, 4°C
log CFU/g
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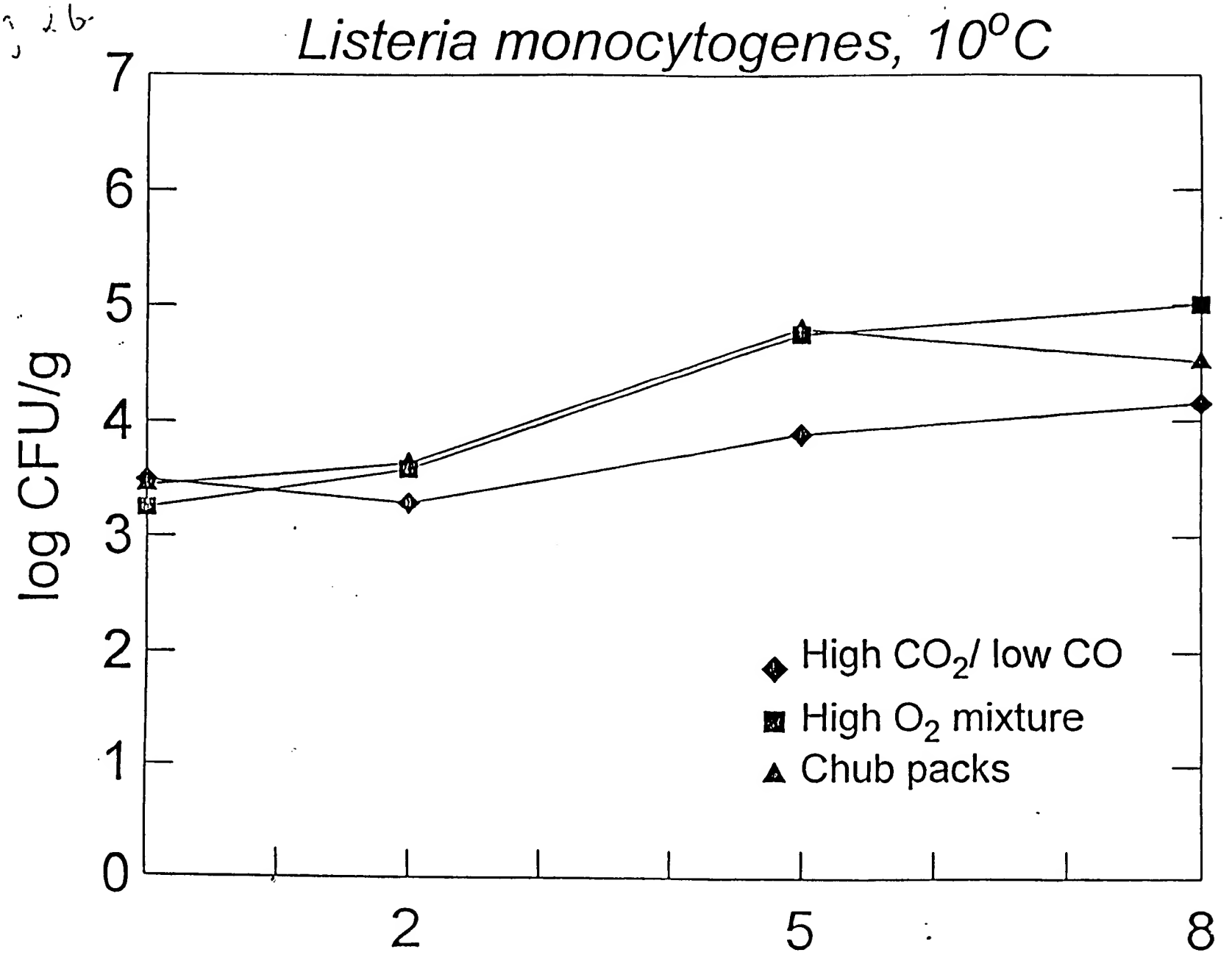
1
2
3
4
5
6
7
2
5
8
14
High CO2/ low CO
High O2 mixture
Chub packs

Fig 2b



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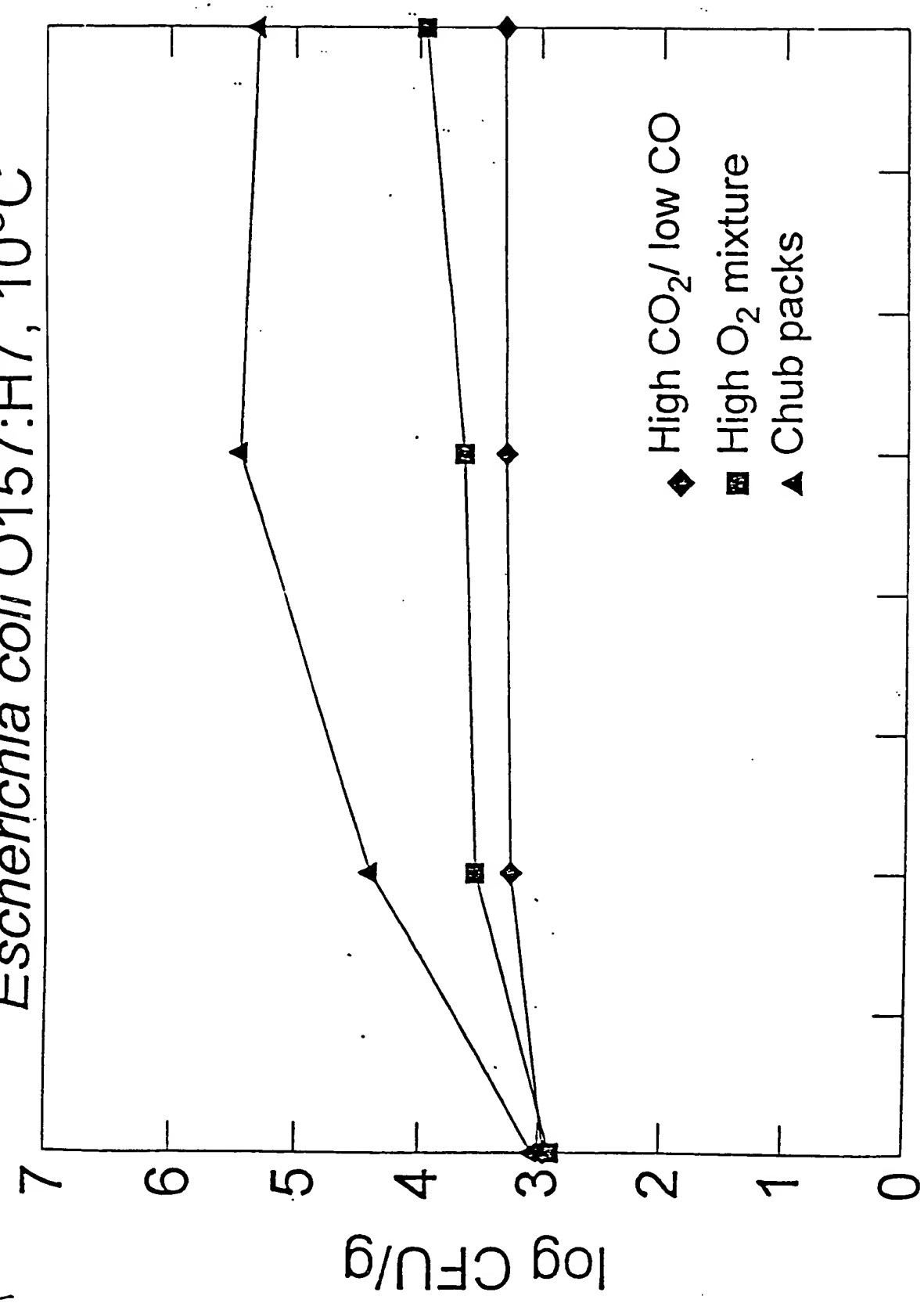
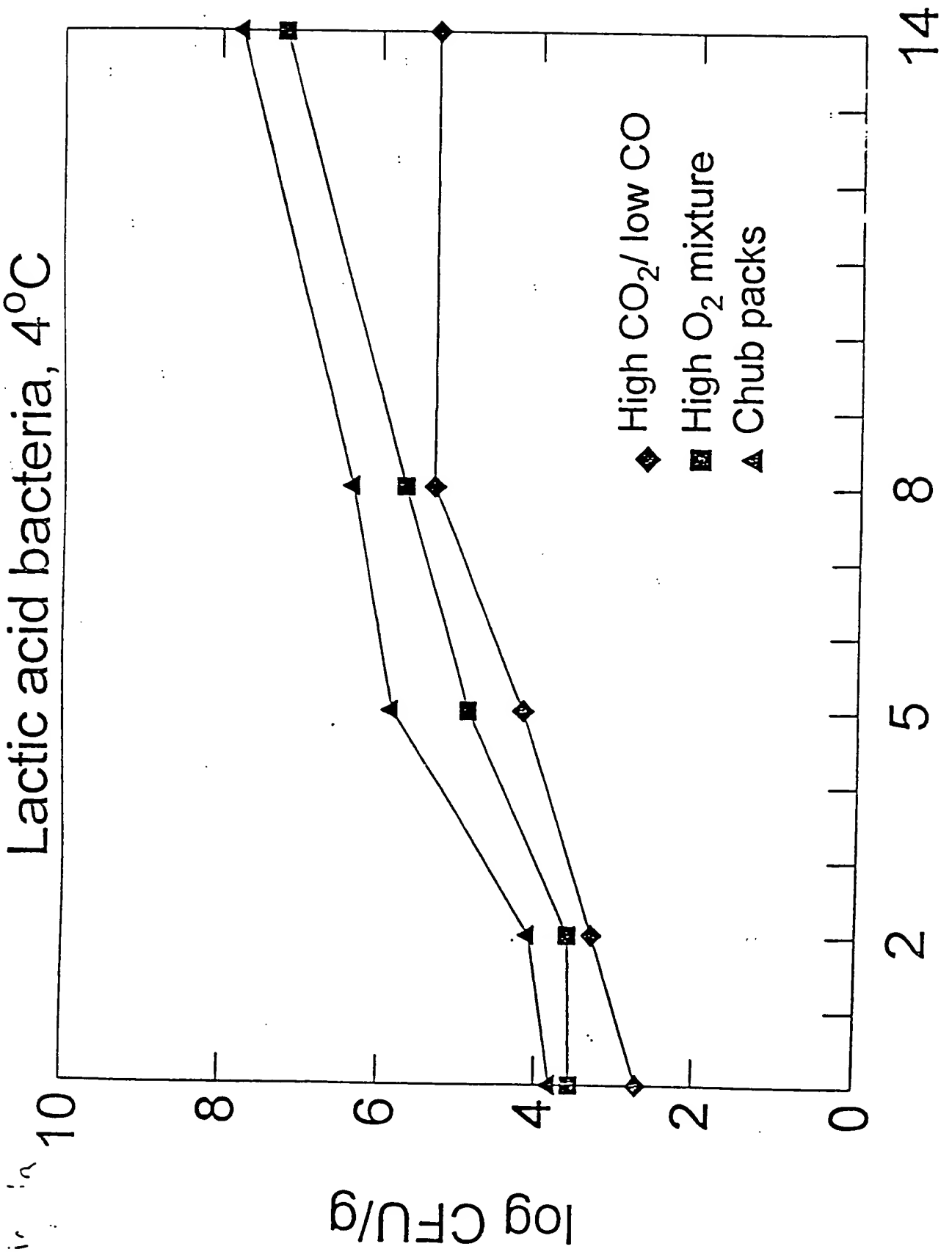

Escherichia coli O157:H7, 10°C
log CFU/g
0
1
2
3
4
5
6
7
2
5
8
High CO_2/ low CO
High O_2 mixture
Chub packs

Fig. 3


Lactic acid bacteria, 4°C
log CFU/g
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2
4
6
8
10
2
5
8
14
High CO_2/ low CO
High O_2 mixture
Chub packs

Fig. 4b

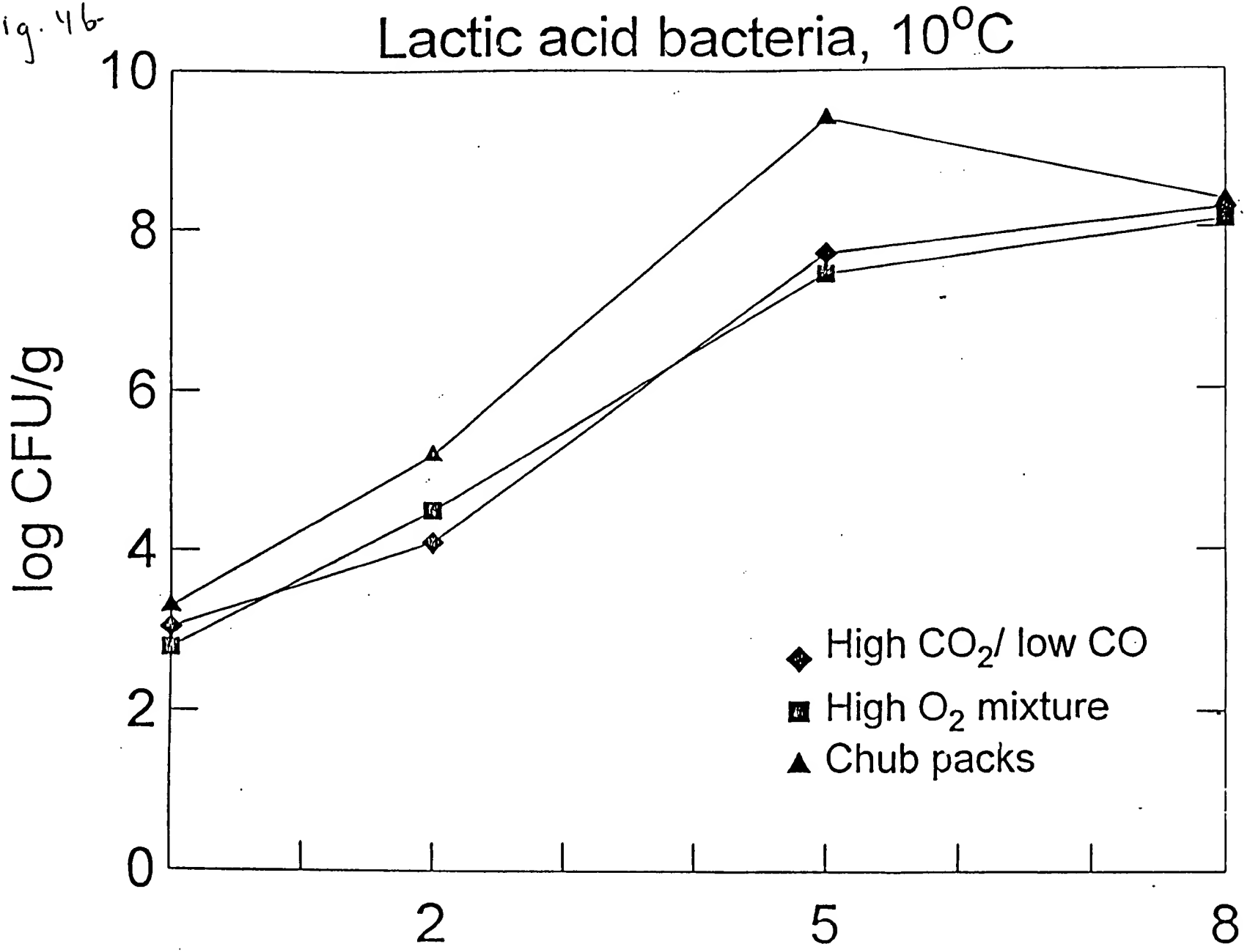

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Fig. 5a

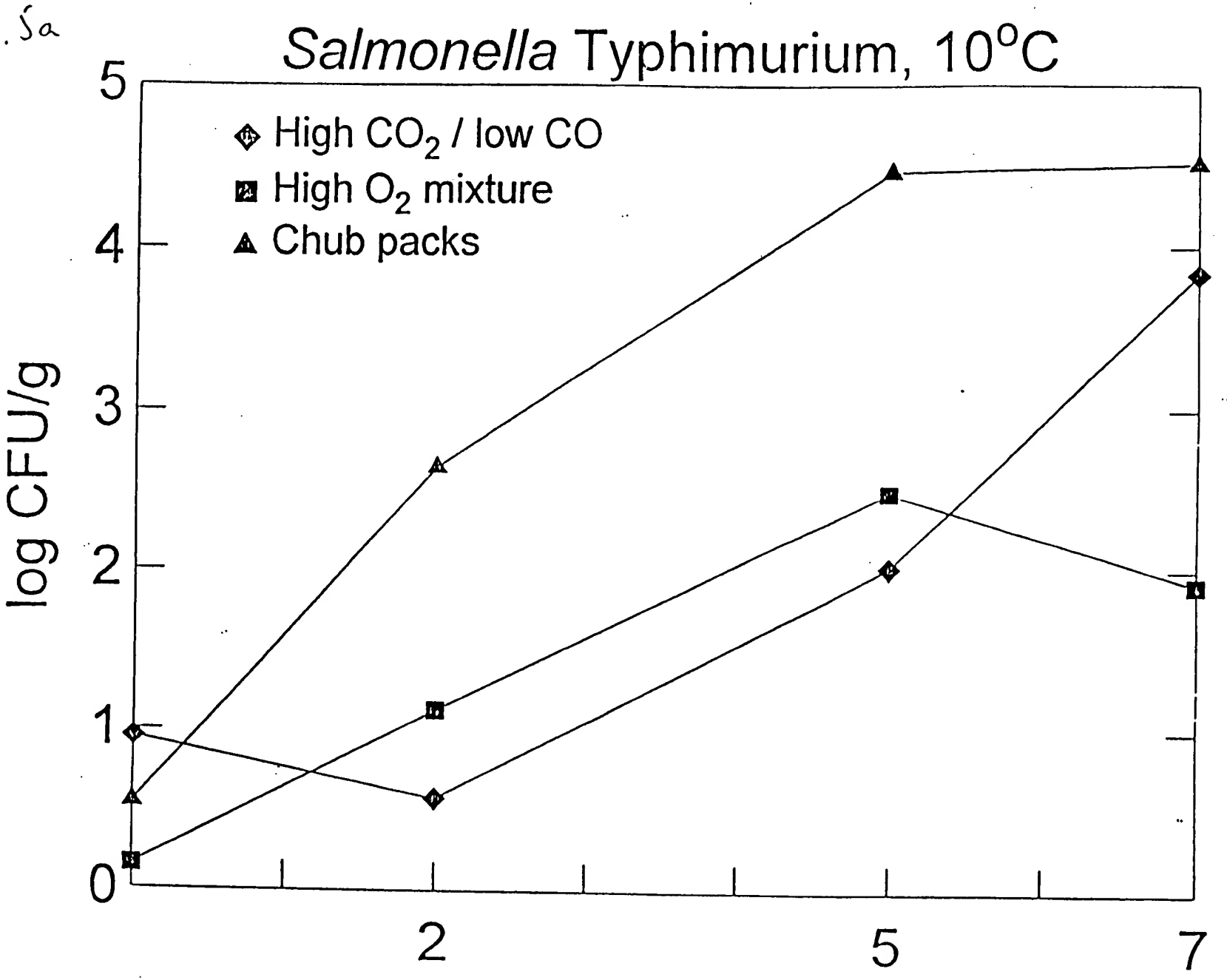

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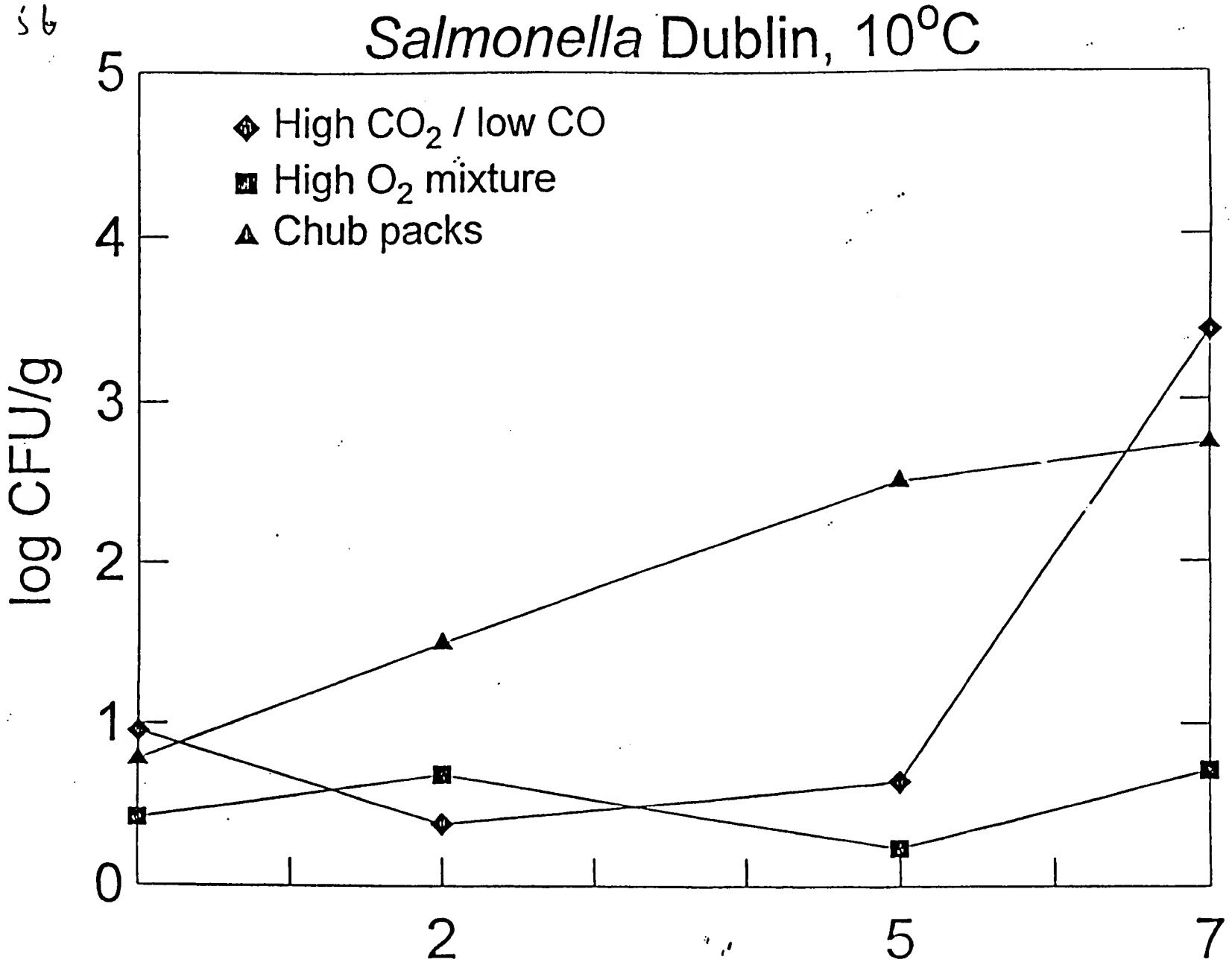
Fig. 5b



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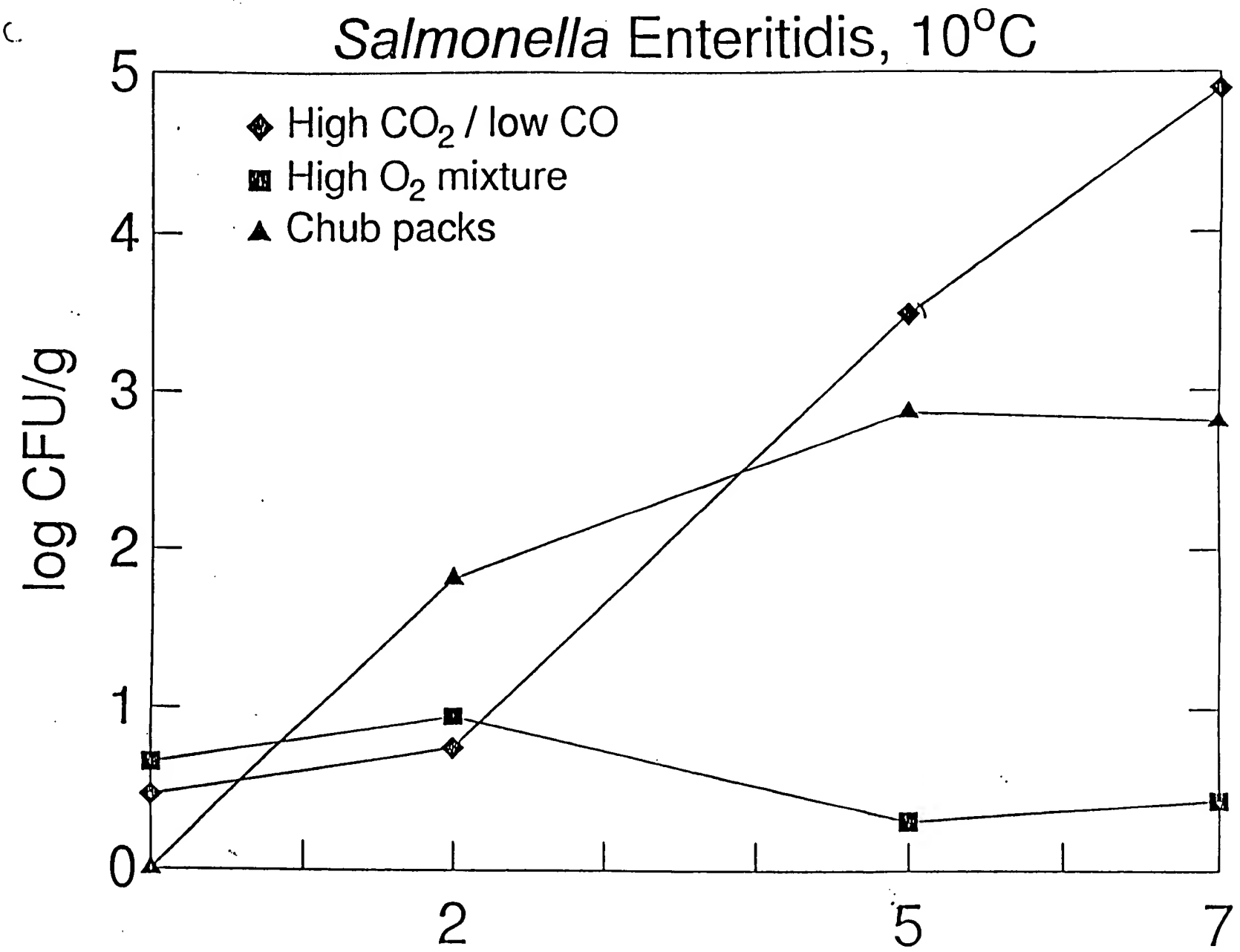
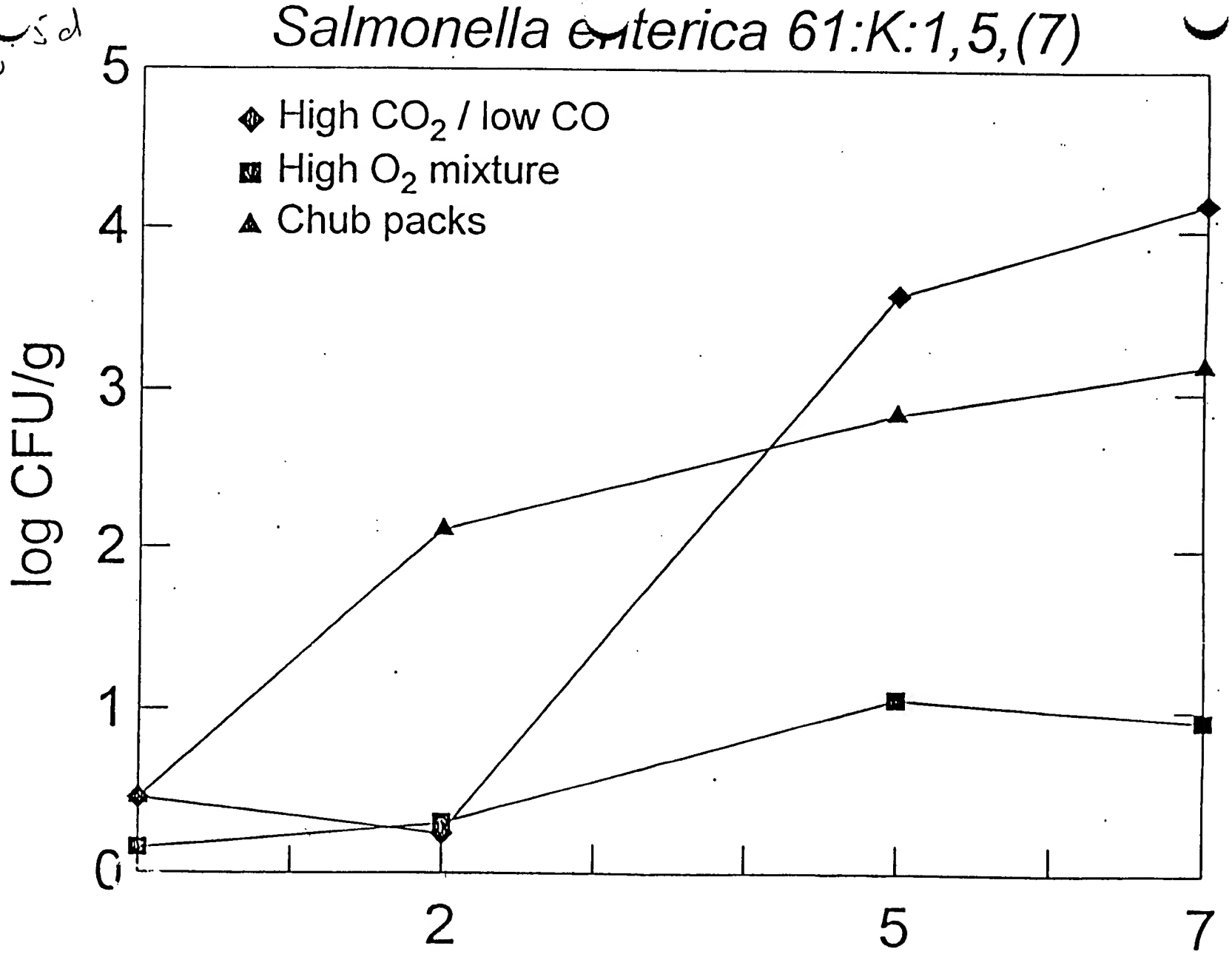
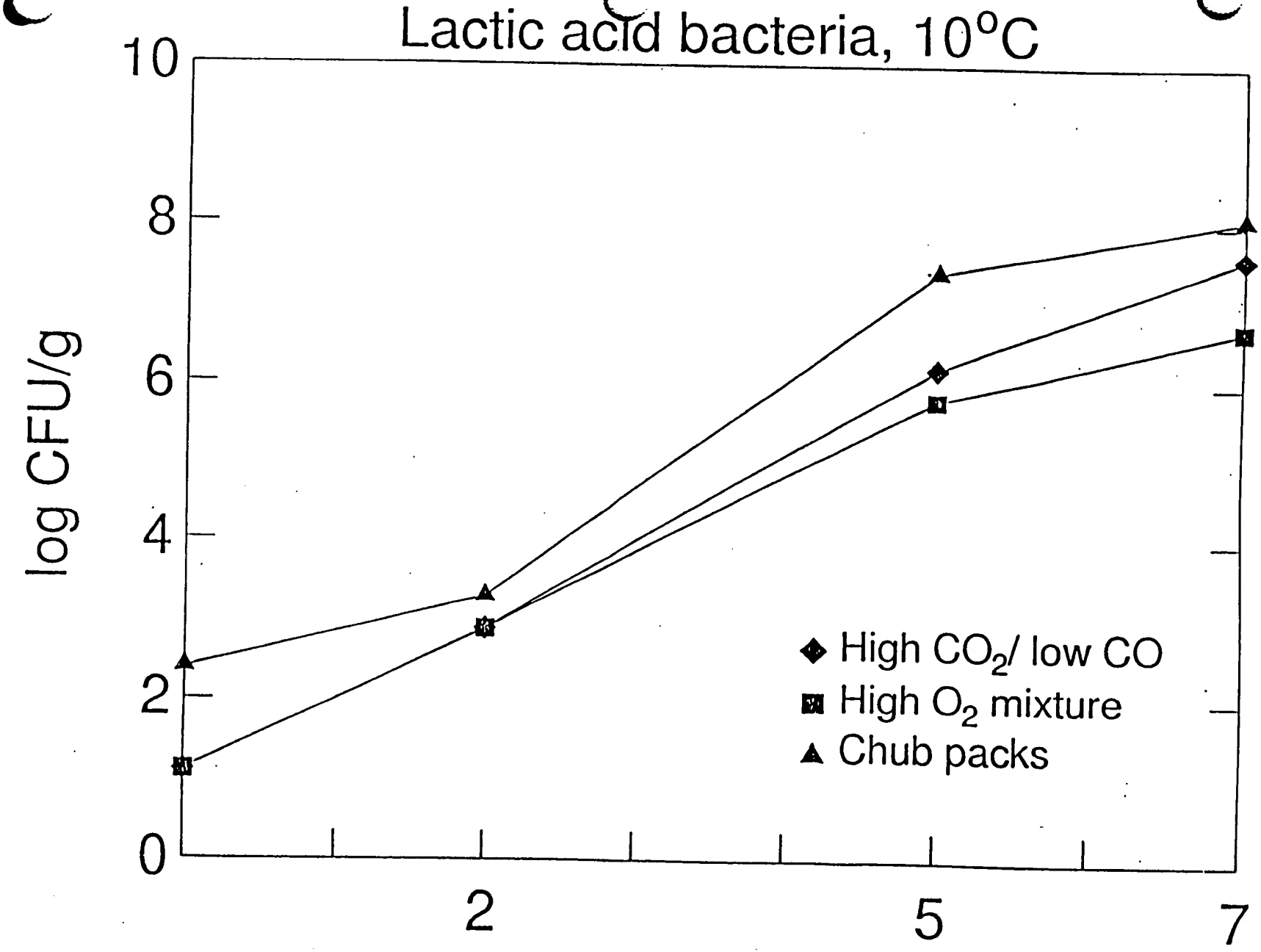

Salmonella Enteritidis, 10°C
High CO_2 / low CO
High O_2 mixture
Chub packs
log CFU/g
0
1
2
3
4
5
2
5
7

000103


Salmonella enterica 61:K:1,5,(7)
High CO_2 / low CO
High O_2 mixture
Chub packs
log CFU/g
0
1
2
3
4
5
2
5
7


Lactic acid bacteria, 10°C
log CFU/g
10
8
6
4
2
0
2
5
7
High CO_2/ low CO
High O_2 mixture
Chub packs

(2)

FOOD MICROBIOLOGY AND FOOD SAFETY INTO THE NEXT MILLENNIUM

Proceedings of the
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Edited by
A.C.J. Tuijtelaars[1]
R.A. Samson[2]
F.M. Rombouts[3]
S. Notermans[1]

[1]TNO Nutrition and Food Research Institute, P.O.Box 370, 3700 AJ Zeist, The Netherlands
[2]CentraalBureau voor Schimmelcultures, P.O.Box 273, 3740 AG Baarn, The Netherlands
[3]Wageningen University and Research Centre, Bomenweg 2, 6703 HD Wageningen, The Netherlands

misinterpreted in the absence of storage trials or in trials of short duration such as 14 days. If *Brochothrix thermosphacta* is a problem organism in a processing facility or on a particular type of meat, lysozyme, Chrisin or mixtures of the two could be used to control its growth during refrigerated anoxic storage.

PACKAGING OF GROUND BEEF IN AN ATMOSPHERE WITH LOW CARBON MONOXIDE AND HIGH CARBON DIOXIDE RESTRAINS GROWTH OF *ESCHERICHIA COLI* O157:H7, *LISTERIA MONOCYTOGENES, YERSINIA ENTEROCOLITICA* AND *SALMONELLA DIARIZONAE*

Nissen H., Alvseike O.[1,2], Bredholt S., Holck A. and Nesbakken T.[2]
MATFORSK, Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway,
[1] Norwegian College of Veterinary Medicine, P.O. Box 8146 Dep., 0033 Oslo Norway, Norwegian Meat Research Centre, P.O. Box 396, 0513 Oslo, Norway
Fax: +47 6497 0333, E-mail: hilde.nissen@matforsk.no

Ground beef for retail sale is most often ready packed in modified atmosphere (MA) or in chub packs. MA packed ground beef prolongs the microbiological shelf life and also maintains an attractive red colour. For the past decade the Norwegian meat industry has been using a gas mixture of 0.3-0.5% CO, 60-70% CO_2 and 30-40% N_2 (the CO comes ready mixed in the N_2). The reason for adding CO to the gas mixture is that it will produce a long-lasting cherry-red colour of the meat (Sørheim et al 1999). The most commonly used gas mixture for retail-ready meat in other European countries is 70% O_2/30% CO_2 (Gill 1996). The high oxygen concentration is needed to keep the red colour of the meat. It is therefore only possible to obtain half the CO_2 concentration used in the CO gas mixture. The microbiological shelf life will be longer than in air, but less than in the CO gas mixture (Sørheim et al 1999).

The inclusion of CO is controversial because the stable cherry–red colour can last beyond the microbiological shelf life of the meat and thus mask spoilage (Kropf 1980). However, the consumer is able to evaluate the microbiological conditions of the meat by off-odours and the shelf life based on odour is significantly longer in the CO mixture only at 4°C. Thus, extended shelf life does not necessarily imply an increased risk of growth of pathogens. In the present study we wanted to compare growth of the pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes*, Yersinia *enterocolitica* and *Salmonella diarizonae*, in ground beef packed in a commercial Norwegian 0.4% CO/60% CO_2/40% N_2 mixture with growth in a high O_2 (70% O_2/30% CO_2) gas mixture and in ground beef in chub packs during storage at 4 and 10°C.

Commercial packages of ground beef (500 g) stored at 10°C were inoculated with the pathogens *Escherichia coli* O157:H7, *L. monocytogenes*, *Y. entercolitca* and *S.*

diarizona, and the ground beef stored at 4°C with *L. monocytogenes* and *Y. enterocolitica*. The inocula of *L. monocytogenes* and *Y.* enterocolitica were cocktails of 3 stationary-phase, rifampicin-resistant strains, the inoculum of *E. coli* O157:H7 was one non-toxic nalidixic/streptomycin resistant strain and that of *S. diarizonae* was a cocktail of 3 strains that were not made antibiotic resistant (plated on selective media for *Salmonella* spp.). Controls of ground beef without inoculated pathogens were stored at both temperatures.

After 5 days storage at 10°C the ground beef packed in the CO mixture had an acceptable smell while beef the packed in the high O_2 mixture and the chub packs had a slight off-odour. After 8 days storage there was a strong off-odour for all the treatments. At 4°C the smell was still acceptable after 14 days of storage in the CO mixture, but the high O_2 mixture and the chub packs had some off-odours. The growth of pathogens was restrained in all samples that had been packed in the gas mixture containing CO. Thus, growth of *Y. enterocolitica* was nearly totally inhibited both at 4 and 10°C, while the number in the samples packed in the high O_2 mixture increased from about $5x10^2$ bacteria per g at day 0 to about 10^4 at day 5 at 4°C and to 10^5 at 10°C. The number in the chub packs were even higher. *L. monocytogens* showed very little growth at 4°C in all of the treatments. At 10°C there was slow growth (from about $5x10^3$ bacteria/g to about 10^4 at day 5) in the CO mixture while the number in the high O_2 mixture and the chub packs were about 10 times higher. Growth of *E. coli* O157:H7 at 10°C storage was slow both in the CO-mixture and the high O_2 mixture. Growth in the chub packs was higher reaching 10^5 bacteria/g on day 5. The growth of *S. diarizonae* followed the same pattern as *E. coli* O157:H7.

Ground beef is a high-risk product because pathogens may be mixed into the product which may not be properly heated before being eaten. The present study shows that the reduced background flora of beef packed in the CO mixture did not result in increased growth of the pathogens. This was probably due to the high concentration of CO_2 in this mixture which particularly inhibits Gram negative bacteria. The O_2 content in the CO mixture was virtually zero throughout storage at both temperatures. At 10°C the O_2 content in the high O_2 gas mixture decreased from 70% to about 35% after 8 days, probably due to aerobic bacterial metabolism. The chub packs had air- permeable casing which probably was the cause of the high bacterial growth in these packs.

The conclusion of the present study is that for the conditions studied, the risk of growth of the pathogens *Y. entrocolitica, L. monocytogenes, E. coli* O157:H7 and *S. diarizonae* in ground beef stored in CO gas mixture is the same as or less than in the ground beef stored in high O_2 or under vacuum (chub packs).

References

Gill, C.O. (1996) Extending the storage life of meats. Meat Science. 43 (Suppl), S99-S109.

Kropf, D.H. (1980). Effects of retail display conditions on meat colour. Proceedings of the Reprocial Meat Conference, 33 15-32.

Sørheim O., Nissen H., Nesbakken T. (1999) The storage of beef and pork packaged in an atmosphere with low carbon and high carbon dioxide. Meat Science 52, 157-164.

English summary

(3)

CONSUMER PURCHASE PROBABILITY OF BEEF AND PORK PACKAGED IN DIFFERENT ATMOSPHERES

Ragnhild Solheim, MATFORSK, Norwegian Food Research Institute, Oslovn. 1, 1430 Ås, Norway

Ground beef, beef loin steaks and pork chops were packaged in modified atmospheres of 0.4% CO/ 60% CO_2/ 40% N_2 (high CO_2/low CO mixture) and 70% O_2/ 30% CO_2 (high O_2 mixture). In addition ground beef was packaged in clipped chub packs, beef loin steaks were vacuum packaged, and pork chops were packaged in an atmosphere of 60% CO_2/ 40% N_2 with each pack containing an O_2 absorber. The purchase probability data were collected by interviewing 126 consumers usually purchasing meat and meat products. The consumers visually compared the samples within each type of meat. The consumers preferred ground beef packaged in the high CO_2/low CO mixture or the high O_2 mixture compared to ground beef packaged in clipped chub packs. Purchase probability increased when pork chops were packaged in the high CO_2/low CO mixture. Pork chops in packs containing an O_2 absorber, were rated lowest in purchase probabilities. The purchase probability for beef loin steaks was similar when packaged in the high CO2/low CO mixture or the high O2 mixture, and these products were preferred compared to beef loin steaks packaged in vacuum.

COMMISSIONED REPORT

MATFORSK – Norwegian Food Research Institute

Osloveien 1, N-1430 Ås
Tel: (+47) 64 97 01 00

Report No.: O-7224
Availability: Confidential

Report Title: Consumer Survey of Meat Products	Date: July 11, 1996
Project Manager/Author: Ragnhild Solheim	Signature of Project Manager: [Signature]
Head of Department: Bjørg Egelandsdal	Signature of Head of Department: [Signature]
Department: Analysis Methodology	Project No: O-7224 FBT
Commissioned by: Norsk Kjøtt [Norwegian Meat]	Commissioner's contact: Truls Nesbakken

Summary/Abstract:

The consumers' (N=126) purchasing tendency for ground meat, pork chops and top loin of beef packaged in various ways was measured by means of a central location test. The consumers indicated their purchasing tendency on a verbal five-point scale from "Will definitely not buy" to "Will definitely buy." Moreover, the consumers estimated their buying frequency for the three different products, and provided their age and gender.

The consumer population was composed of 62 % women and 38 % men. The age distribution was roughly equivalent for both genders.

Purchasing Frequency:
The major segment (47.6 %) of the participants said that they buy ground meat two to three times a month, while 20.6 % buy ground meat once a month.

Pork chops were purchased two to three times a month by 29.4 % of the participants, while 38.1 % bought pork chops once a month. 29 % of the participants bought pork chops less frequently than once a month.

Top loin was purchased less frequently than once a month by 58.1 % of the participants in the study. 18 % said that they buy top loin once a month, and 19.4 % indicated that they buy top loin once a week.

Purchasing Tendency:
Ground meat: Ground meat packaged with CO gas received the same average score for purchasing tendency as ground meat packaged in O_2 gas, while ground meat packaged as sausage had the lowest score for purchasing tendency.

Pork Chops: Pork chops packaged in CO gas received the highest total score for purchasing tendency, while pork chops packaged in O_2 gas received the second highest total score and pork chops packaged with oxygen absorber received the lowest score.

Top Loin: Top loin packaged in CO gas and in O_2 gas had roughly the same average score for purchasing tendency, while vacuum-packaged top loin received the lowest score for purchasing tendency.

Table of Contents

1. Premise

The consumers' choice of meat products may be attributed to factors such as how the products are presented in their packaging. The consumers' purchasing tendency for meat products packaged in accordance with various principles was therefore measured.

2. Implementation

2.1 Materials and Survey Conditions

The survey was taken at Drøbak City [shopping center] on June 12, 1996 (Tuesday) from 10 am to 4 pm.

Ground meat, pork chops and top loin of beef packaged in accordance with various principles (Table 1) was presented to consumers who eat these types of products. The products were delivered to MATFORSK on June 8, 1996 and stored at 4°C until the survey date. Products from the same group were placed side by side on a table under lighting with a strength of approximately 2000 lux (equivalent to the light intensity of a refrigerated meat counter in a store). The products were replaced with cold stored products every three hours.

Table 1. Packaging methods for meat products tested in consumer survey

Meat Product		Packaging Method			
	Sausage	CO	O_2	Vacuum	O_2 w/absorber
Ground Meat	x	x	x		
Pork Chops		x	x		x
Top loin of beef		x	x	x	

x = packaging method used

2.2 Method

The products were coded with three-digit random numbers and evaluated in a systematically rotating order. The consumers indicated purchasing probability on a verbal scale and purchasing frequency for the product, and gave their age and gender (Figure 1). Following the evaluation, the verbal scale was translated into numerical values from 1 to 5, where 1=Will definitely not buy, and 5=Will definitely buy. The consumers spent between 5 and 10 minutes answering the questions.

MATFORSK – Norwegian Food Research Institute

Dear Consumer!
We are taking a survey on the consumer opinion concerning a selection of meat products as they appear in the meat counter. We hope that you will take a minute to let the meat producers know your opinion!

What to do:
1. Please take a look at the samples of top loin.
2. Consider whether you would buy these products the way they appear, assuming they are priced the same. Evaluate the products in the order listed below and check one box for each product.

Sample marked 763		Sample marked 288	
Will definitely buy	☐	Will definitely buy	☐
May buy	☐	May buy	☐
May/may not buy	☐	May/may not buy	☐
May not buy	☐	May not buy	☐
Will definitely not buy	☐	Will definitely not buy	☐

Sample marked 911	
Will definitely buy	☐
May buy	☐
May/may not buy	☐
May not buy	☐
Will definitely not buy	☐

In closing please answer the questions below about your age, gender and how often you buy top loin of beef.

My age (check one):

18–25 years old	☐
26–35 years old	☐
36–45 years old	☐
46–55 years old	☐
56–65 years old	☐
over 65 years old	☐

Gender (check one):
Female ☐ Male ☐

I buy top loin of beef (refrigerated):

Less than once a month	☐
Once a month	☐
Two to three times a month	☐
Once a week	☐
More than once a week.	☐

THANK YOU FOR YOUR ASSISTANCE!

Figure 1. Questionnaire for consumer survey of meat products. Corresponding forms were used for pork chops and ground meat.

2.3 Consumer Population

124–126 consumers over 18 years of age participated in the survey of the three different meat products. There were a few more women than men among the participants, and there were fewer participants over 56 years of age than in the other age groups (Table 2). The age distribution among men and women was the same.

Table 2. Age and gender distribution among consumers participating in the survey.

	No. of consumers	Gender (%)		Age (year, % distribution)					
Meat Product		Women	Men	18–25	26–35	36–45	46–55	56–65	Over 65
Ground Meat	125	61.6	38.4	15.9	19.8	18.3	20.6	11.1	14.3
Pork Chops	126	61.9	38.1	15.1	18.3	19.8	19.8	11.1	15.9
Top Loin	124	63.7	36.3	14.5	21.0	16.9	21.8	12.9	12.9

3. Results

3.1 Purchasing Frequency for Meat Products

Ground meat was most frequently bought, followed by pork chops, while top loin was rarely bought by the consumers participating in this study (Table 3). There were similar purchasing frequencies in the various age and gender groups.

Table 3. Purchasing Frequency for Three Different Meat Products

	Meat Product		
Purchasing Frequency	Ground Meat (N=125)	Pork Chops (N=126)	Top Loin (N=124)
Less than once a month	11.1	29.4	58.1
Once a month	20.6	38.1	18.5
Two to three times a month	47.6	29.4	19.4
Once a week	15.1	3.2	3.2
More than once a week	5.6	0.0	0.8

3.2 Purchasing Tendency for Meat Products

A detailed overview of the purchasing tendency is provided in Attachment 1.

All differences described in the following were significant to a degree of 95 %.

Ground Meat
Ground meat packaged as sausage was least appreciated by the consumers (see Figure 2). Ground meat packaged in CO gas and ground meat packaged in O_2 gas received the same average score for purchasing tendency. This result occurred regardless of consumer age and gender.

Comments from the consumers:
The wrapping film used to pack meat as sausage hides the contents.

[Above bar chart] **Purchasing Tendency for Ground Meat**

[Beside bar chart] Average score (N=126) [Bar chart]

[Below bar chart] As Sausage CO gas O_2
Packaging Method

Figure 2. Purchasing tendency for meat products. 1=Will definitely not buy, and 5=Will definitely buy the product as presented in the survey.

Pork Chops
Pork chops packaged in CO gas received the highest average score for purchasing tendency, pork chops packaged in O_2 gas received the second highest score and pork chops packaged with oxygen absorber received the lowest average score (see Figure 3). This result occurred regardless of consumer age and gender.

Comments from consumers:
The pork chops packaged with absorber look gray/brown – are they old? expired?

Sample packaged with CO and O_2: nitrite added?

[Above bar chart] Purchasing Tendency for Ground Meat

[Beside bar chart] Average score (N=126) [Bar chart]

[Below bar chart] O_2 absorber CO gas O_2
Packaging Method

Figure 3. Purchasing tendency for meat products. 1=Will definitely not buy, and 5=Will definitely buy the product as presented in the survey.

Top Loin
Top loin packaged in CO gas and in O_2 gas received approximately the same average score for purchasing tendency (see Figure 4). Vacuum packaged top loin received a lower score for purchasing tendency than the two aforementioned samples. Men indicated roughly equivalent purchasing tendencies for the three varieties, while women indicated the highest purchasing tendency for top loin packaged in CO gas, followed by top loin packaged in O_2 gas, and the lowest purchasing tendency for vacuum packaged top loin.

Comments from consumers:
Sample packaged in CO and O_2: nitrite added?

Sample packaged in CO: "artificial" sides.

Vacuum packaged sample: looks as if it has been squeezed.

[Above bar chart] **Purchasing Tendency for Top Loin**

[Beside bar chart] Average score (N=125) [Bar chart]

[Below bar chart] Vacuum CO gas O_2
Packaging Method

Figure 4. Purchasing tendency for meat products. 1=Will definitely not buy and 5=Will definitely buy the product as presented in the survey.

4. Conclusion

Ground Meat: Ground meat packaged in CO gas and ground meat packaged in O_2 gas received the same average score for purchasing tendency, while ground meat packaged as sausage received the lowest score for purchasing tendency.

Pork Chops: Pork chops packaged in CO gas received the highest score for purchasing tendency, pork chops packaged in O_2 gas received the second highest total score, while pork chops packaged with oxygen absorber received the lowest score.

Top Loin: Top loin packaged in CO gas and in O_2 gas received approximately the same average score for purchasing tendency, and vacuum packaged top loin received the lowest score for purchasing tendency.

Comments

This type of survey does not have representative sampling of consumers in terms of the population as a whole or a specific population segment. The make up of the survey represents a model for a purchasing situation. These circumstances must be taken into account when interpreting the results.

MATFORSK – Norwegian Food Research Institute

ATTACHMENT 1

Overview of Results from Consumer Study of Meat Products

	Average	*Standard Deviation*	*Median*
Ground Meat (N=126)			
As sausage	2.5	1.5	2
CO gas	3.7	1.4	4
O_2	3.7	1.4	4
Pork Chops (N=126)			
O_2 absorber	1.9	1.3	1
CO gas	4.6	0.7	5
O_2	3.6	1.4	4
Top Loin (N=125)			
Vacuum	2.9	1.6	3
CO gas	4.2	1.1	4.5
O_2	4.0	1.1	4

Prosjektmedarbeidere:

Ansvarlig for *opplegg og rapport*:	Ragnhild Solheim
Ansvarlig for *prøvemottak og lyssetting*:	Oddvin Sørheim
Ansvarlig for *praktisk gjennomføring*:	Inger Johanne Fjøsne
Øvrige deltakere i gjennomføring:	Asgeir Nilsen Janne Haugdahl Vibeke Høst Oddvin Sørheim
Ansvarlig for *statistisk bearbeiding*:	Per Lea

(4)

English summary

DISCOLORATION OF MEAT AS AN INDICATOR OF LEAKAGES IN PACKAGES CONTAINING A CO GAS MIXTURE

Oddvin Sørheim, MATFORSK, Norwegian Food Research Institute, Oslovn. 1, 1430 Ås, Norway

The aim of the experiment was to study discoloration of meat packaged in a gas mixture of 60 % CO_2/40 % N_2/ 0.4 % CO with different concentrations of residual O_2 added. Tests were performed on ground beef with 1 % NaCl, aged beef loin steaks and pork chops. Leakages were simulated by injecting different amounts of air with a syringe into the packages after two days storage. Discoloration of the meat was measured as reduction in a* (redness) values and evaluated visually. Ground beef had a low tolerance level of residual O_2 because it was discoloured in atmospheres containing more than 1 % O_2. Beef loin steaks and pork chops were slightly discoloured in more than 2 and 5 % O_2, respectively. The results suggest that discoloration can be an indicator of leakages for ground beef, but not for beef loin steaks and pork chops.

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MATFORSK – Norwegian Food Research Institute

Osloveien 1, N-1430 Ås
Tel: (+47) 64 97 01 00

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Project Manager/Author: Oddvin Sørheim	Signature of Project Manager: [Signature]
Head of Department: Astrid Nilsson	Signature of Head of Department: [Signature]
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Summary/Abstract:

Tests were carried out to find the tolerance limits for residual O_2 for discoloration of meat packaged in CO gas mixture with simulated leak. Various concentrations of air were added to the packages of ground meat, top loin and pork chops with a mixture of 60% CO_2 / 40% N_2 / 0.4% CO two days after packaging. Ground meat packaged in gas containing more than 1% O_2 was clearly discolored, while top loin and pork chops, respectively packaged in gas containing more than 2 and 5% O_2 showed only minor discoloration. The results indicate that discoloration can serve as an indicator of leakage for ground meat, but not for top loin and pork chops.

MATFORSK – Norwegian Food Research Institute

Meat Discoloration as an Indicator of Leakage in Packages with CO Gas Mixtures

Purpose

The purpose of the survey was to find the tolerance limits for residual O_2 with regard to discoloration of ground meat, top loin and pork chops packaged in a CO gas mixture.

Implementation of the Study

Samples of ground meat with 1% NaCl (20 pieces), tenderized top loin of beef (18 pieces) and pork chops (14 pieces) were gas packed on a Ilapak Delta 2000 machine (Ilapak, Switzerland) on a tray in BDF 550 shrink film (Cryovac). The gas mixture consisted of 60% CO_2 / 40% N_2 / 0.4% CO. The samples were stored out of light at 4°C. After two days of storage, air was added to the packages to increase the O_2 content, i.e. a simulated leak. This was done by sucking out the gas in the package and replacing it with air by means of a syringe and a septa . 0-2.0% O_2 was added to the ground meat, 0-3.2% O_2 was added to the top loin, and 0-13.9% O_2 was added to the pork chops, in all cases with a spectrum of O_2 concentration in their respective ranges. After the replacement of gases, the concentrations of O2 and CO_2 were measured by means of two Toray instruments, type LC 700F and PG-100 (Toray Eng., Japan). The remaining storage time before unwrapping was 2 days for ground meat and 5 days for both top loin and pork chops.

Upon unwrapping, the O_2 and CO_2 concentrations in the packages were once again measured. Two judges then visually judged the color of the unopened packages according to a scale (1=fresh meat red, 2=dark red, 3=somewhat discolored, 4=moderately discolored, 5=extremely discolored). The packages were then opened, and the color was measured with a Minolta Chroma Meter CR-300 (Minolta Camera Co., Japan) directly on the surface of the meat within 1 minute of opening. The instrument had light source D_{65} at 8 mm aperture, and the color was measured in CIE (1976) L* (luminosity), a* (redness) and b* (yellowness). Lastly, the pH was measured directly in the meat with a Ingold Xerolyt electrode (Mettler-Toledo A.G., Switzerland).

Results and Discussion

The correlation between discoloration upon unwrapping and O_2 concentration when replacing the gas proved to best be expressed by an a* value (redness) and visual color evaluation. Attached are a plot of the a* and O_2 concentration for ground meat, top loin and pork chops; see figures 1, 2, and 3. The correlation coefficients for the three products were calculated to -0.71, -0.33, and -0.51. The relatively low coefficients are partly due to the large spread of the measured values and partly because the correlation between a* and O_2 does not appear to be linear.

For ground meat we found a reduction of approximately 4-5 a* values from 0 to 1% O_2. A reduction of a* to this degree is readily apparent. Samples stored in 1% or higher levels of O_2 had a score of between 3 and 4 on the color scale, i.e. slight to moderate discoloration. The results indicate that the tolerance limit for discoloration of ground meat in CO mixture is approximately 1% O_2.

For top loin, on the other hand, a smaller decrease in a* and a weak correlation between a* and O_2 concentration were found. However, there seemed to be a reduction of about 2 a* values between 0 and 2% O_2, with a color scale score of about 3 on samples stored in over 2% O_2. This indicates a weak discoloration with a tolerance limit of approximately 2 % O_2 for top loin.

The color of pork chops proved to be only slightly affected by the O_2 concentration in the packaging gas, even when up to 2/3 of the gas was replaced with air. We found a reduction of 1 to 1.5 a* values between 0 and 5% O_2 in the package gas, but this barely registered as discoloration with a score of 2-3 on the color scale.

The pH values at the end of the leak test were on average 5.59, 5.62 and 5.42 for ground meat, top loin and pork chops respectively.

Between the start and the end of the leak tests, we measured a reduction in the O_2 concentrations of 80, 40 and 30 % for ground meat, top loin and pork chops respectively. This reduction can be due to meat respiration or consumption of O_2 by bacteria. Ground meat has a high consumption of O_2 due to a large surface area exposed to surrounding gas and frequently higher bacteria counts than whole meat.

The significance of residual O_2 in package gas with regard to discoloration and microbiological storage life has been discussed previously in the report "Fresh Meat in Consumer Packaging - an Evaluation of Various Packaging Methods and Their Effect on Meat Quality." For storage in gas containing CO_2 and/or N_2 without the presence of CO, tolerance limits for discoloration have been found to be below 0.1 and 0.5% O_2 for beef and pork respectively. Tests on pork has shown that the microbiological storage life was reduced when the package gas contained more than 2-4% O_2.

The ground meat containing 1% sodium that was tested in this survey, had obvious discoloration when the CO mixture contained at least 1% O_2. Sodium functions as a pro-oxidant, and will usually intensify or accelerate the discoloration of the meat. It is therefore likely that consumers will react on the color of ground meat when small leaks in the packaging exist. For top loin and pork chops, however, there is little likelihood that the minor discoloration occurring at above 2 and 5% O_2 will serve as an indicator of leakage to the regular consumer. The lighting in store refrigerating counters will often conceal minor color nuances. All in all, these results show that CO has a strong bond to the myoglobine in whole, unsalted meat, which prevents the carbon myoglobine from being destabilized by O_2 in the gas. Hence, discoloration is not a good indicator with regard to alerting consumers of leaks and risk of increased bacterial growth in meat such as top loin and pork chops.

Thanks
We are grateful to Frank Lundby for his valuable technical assistance.

[Left-hand side of graph] a* [Plot]

[Below graph] % Oxygen

Figure 1 The correlation between a (redness) and O_2 concentration for ground meat packaged in a mixture of 60% CO_2 / 40% N_2 /0.4% CO after two days storage at* 4°C. n=20, r=-0.71.

[Left-hand side of graph] a* [Plot]

[Below graph] % Oxygen

Figure 2 The correlation between a (redness) and O_2 concentration for top loin packaged in a mixture of 60% CO_2 / 40% N_2 /0.4% CO after five days storage at* 4°C. n=18, r=-0.33.

[Left-hand side of graph] a* [Plot]

[Below graph] % Oxygen

Figure 3 The correlation between a (redness) and O_2 concentration for pork chops packaged in a mixture of 60% CO_2 / 40% N_2 /0.4% CO after five days storage at* 4°C. n=14, r=-0.51.

(5)

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Review

Technological, hygienic and toxicological aspects of carbon monoxide used in modified-atmosphere packaging of meat

Oddvin Sørheim, Tore Aune and Truls Nesbakken

Retail meat can be packaged in gas mixtures containing 60–70% carbon dioxide (CO_2), 30–40% nitrogen (N_2) and <0.5% carbon monoxide (CO). This gas mixture with CO provides a unique combination of a long microbiological shelf life and a stable, cherry red colour of the meat. The shelf life of meat packaged in the CO mixture is longer than that of meat packaged in the commonly used atmospheres with high oxygen (O_2), that is, approximately 70% O_2 and 30% CO_2. The consumption of meat that has been packaged in a CO mixture will result in only negligible levels of carboxyhaemoglobin in the blood. It is highly improbable that the use of CO in the packaging of meat will present a toxic threat to consumers.

Modified-atmosphere packaging (MAP) is gaining increasing application in modern food distribution. Meat intended for retail sale can either be wrapped in vapour-tight, oxygen-permeable films or packaged in gas-tight films with a modified atmosphere (MA). The main purposes of the MAP of meat are twofold: to ensure the microbiological shelf life and the attractive red colour of the product. Consumers frequently interpret the colour of meat on retail display as an indicator of wholesomeness[1].

CO is a colourless, odourless and tasteless gas. It is produced mainly through incomplete combustion of carbon-containing materials. Natural background levels of CO are 0.01–0.9 mg/m³ (Ref. 2). In urban areas, 8-h mean concentrations of CO (i.e. mean CO concentrations are measured for each possible 8-h interval during a 24-h period, then averaged) are generally <20 mg/m³; however, maximum 8-h concentrations (i.e. the maximum mean concentration found during any one 8-h period) of up to 60 mg/m³ have been recorded[2]. By far the most common cause of elevated CO concentrations in the blood is tobacco smoking[3].

A challenge in the MAP of retail meat is the stabilization of the red colour of the product. The positive effect of CO on meat colour was known and patented over 100 years ago[4]. Despite this knowledge, CO has to date been applied commercially only to a limited extent in the MAP of meat. During the past 10 years, the Norwegian meat industry has been using a gas mixture of 60–70% CO_2, 30–40% N_2 and 0.3–0.4% CO for the packaging of fresh retail meat, namely beef, pork and lamb. This gas mixture with CO maintains a stable, cherry red colour combined with a long microbiological shelf life of the meat. The market share of retail meat packaged in this CO mixture in Norway is estimated at 50–60% (Dag Hallan, pers. commun.). In addition, some meat is also initially bulk-packaged in the CO mixture, and thereafter repackaged on trays with O_2-permeable films in retail outlets. In other European countries not using such CO mixtures, market shares of retail meat packaged in atmospheres with a high O_2 concentration, with considerably shorter shelf lives, have been reported to be only 10–40%[5,6].

In this article, we have evaluated the toxicological aspects of CO, and its mode of action and application in the MAP of meat.

Technological and hygienic aspects of CO as a packaging gas for meat

Gases for MAP

The most commonly used gases for the MAP of meat are CO_2, N_2 and O_2, although other gases, including CO, nitrous oxide, argon and ozone, have been tried to a limited extent[4]. CO_2 inhibits the growth of many microorganisms, but it has no effect *per se* on the colour of meat[6]. CO_2 is absorbed in meat and fat tissue at a ratio of ~1 litre of gas per kg of tissue[7]. N_2 affects neither the microbiology nor the colour of the meat, but prevents packages from collapsing, because it is not absorbed by the product. O_2 supports the growth of aerobic microorganisms; thus, removal of O_2 from the MA will extend the microbiological shelf life. High O_2 concentrations cause meat to have a temporary bright red colour; oxygen binds to the muscle pigment myoglobin, forming oxymyoglobin, which is gradually oxidized to grey-green-brown metmyoglobin[8] (Fig. 1). Gases for the packaging of meat are seldom used alone, but in mixtures, which vary according to the application. Examples of different gas mixtures for the MAP of meat are discussed below.

Oddvin Sørheim is at MATFORSK – Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway (fax: +47-64-970333; e-mail: oddvin.sorheim@matforsk.no). Tore Aune is at the Department of Pharmacology, Microbiology and Food Hygiene, Norwegian College of Veterinary Medicine, PO Box 8146 Dep., N-0033 Oslo, Norway. Truls Nesbakken is at the Norwegian Meat Cooperative, Department of Research and Development, PO Box 360 Økern, N-0513 Oslo, Norway; and at The Royal Veterinary and Agricultural University, Department of Veterinary Microbiology, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark.

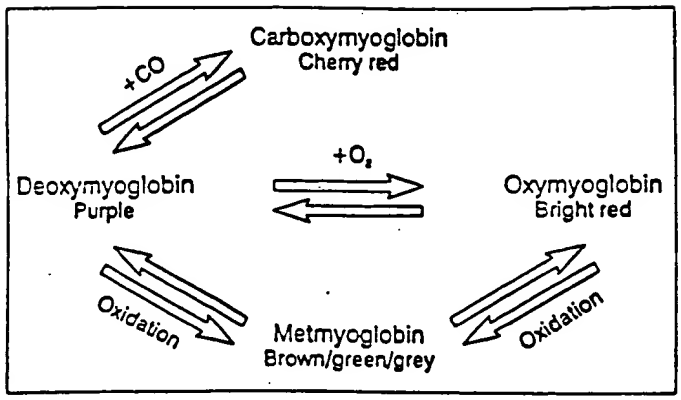

Fig. 1
Myoglobin forms and colour of meat.

CO and colour

The main function of low levels of CO in MAs is to give meat a stable, cherry red colour, as a result of strong binding of CO to myoglobin and the formation of carboxymyoglobin[9] (Fig. 1). Although a substantial increase in the shelf life of meat can be obtained by using various MAs, it is often limited by discolouration due to the oxidation of myoglobin to metmyoglobin. This discolouration can be prevented by the inclusion of a low level of CO in the gas mixture.

Carboxymyoglobin is more resistant to oxidation than oxymyoglobin, owing to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule[10]. CO at concentrations of 1–5% increased the reduction of metmyoglobin, even in the presence of air[11].

Examples of different gas mixtures that contain CO for the packaging of meat are given in Table 1. A mixture of 2% CO and 98% air was very effective in stabilizing the colour of beef for 15 d, compared with 5 d in air alone[12]. Ground beef patties stored in an atmosphere of 1% CO/50% CO_2/49% air retained a stable, red colour for at least 6 d, whereas the colour of samples stored in air was stable for only 3 d[13].

The colour of beef was analysed during storage in MAs containing N_2 with 0.5–10% CO. Levels of CO >0.5% resulted in a stable, red colour for >30 d, whereas discolouration occurred after 5 d storage in control samples packaged in air[14]. In addition, samples of this beef were exposed to pure CO for 2–16 h before packaging in air. The colour stability of the CO-treated samples was no greater than that of untreated samples. However, in other experiments the exposure of beef to CO before vacuum packaging increased its redness and colour acceptability during subsequent chilled or frozen storage[9,17]. Beef loin roasts stored in 1% CO/51% CO_2/30% O_2/18% N_2 were shown to have lower levels of metmyoglobin on their surface than vacuum-packaged roasts. After a further 4 d on retail display, steaks from the roasts underwent less discolouration if they had previously been stored in the CO mixture[15]. Ground beef and beef loin steaks packaged in 1% CO/50% CO_2/25% N_2/24% O_2 or 1% CO/20% CO_2/9% N_2/70% O_2 retained a stable colour for 29 d[16]. Similarly, beef loin steaks packaged in 0.4% CO/60% CO_2/40% N_2 maintained a stable, cherry red colour for up to 22 d[18]. Experiments with beef and higher levels of CO, that is, 2% CO/20% CO_2/78% N_2, resulted in meat that had a stable colour; however, its colour was characterized as 'too artificial' by a sensory panel[6].

Based on the cited literature, the presence of 0.4–1.0% CO in MAs used for the packaging of meat seems sufficient to produce a stable, cherry red colour.

Cooked, cured meat products can also benefit from storage in MAs containing CO. Packaging in 1% CO/99% N_2 stabilized the colour of sliced bologna, indicating binding between CO and denatured myoglobin[19].

Under certain circumstances, an undesirable pink or red colour can arise in cooked white meat, such as poultry, and cooked meat products without added nitrite[20]. Such colour problems can sometimes be linked with exposure to CO, which results in similar colours occurring after the use of MAs with CO. For example, roasted turkey was noted to be pink; this was probably due to the presence of CO and nitric oxide in the combustion gases in the oven. The pink colour did not occur when the turkeys were roasted in complete isolation from the oven gases[21]. Combustion engines produce various gases, including CO, which can affect live poultry during transportation to the abattoir. Meat from chickens that were exposed to exhaust fumes immediately before slaughter developed an undesired red colour on cooking[22].

Table 1. Applications of carbon monoxide (CO) in the modified-atmosphere packaging of meat

Gas combinations (%)					
CO	CO_2	N_2	O_2	Air	Refs
2				98	12
1	50			49	13
0.5–10		90–99.5			14
1	51	18	30		15
1	50	25	24		16
1	20	9	70		16
2	20	78			6
1–5				95–99	10
100[a]					9, 14, 17
0.4	60	40			18
0.3–0.4	60–70	30–40			[b]

[a]Exposure before packaging
[b]Data supplied by Norwegian meat plants

CO and microbiology

Generally, the purpose of most of the experiments investigating the use of CO as a small component of MAs for meat has been to study its effect on colour stability, and more seldom its microbiological aspects. The growth of psychrotropic bacteria on beef stored in MAs containing 0.5–10% CO in N_2 was lower, relative to controls, resulting in an increase in odour shelf life at

temperatures in the range of 0–10°C[14]. For example, beef packaged in a MA of 1% CO/99% N_2 had an odour shelf life of 24 d, compared with 18 d in 100% N_2, and 7 d in air at 5°C. However, in another experiment with a MA of 20% CO_2/70% O_2/9% N_2, the addition of 1% CO had no effect on the microbiological growth on ground beef and beef steaks[16]. The presence of bacteriostatic CO_2 in the latter experiment apparently reduced the importance of the effect of CO on the shelf life of the meat. The odour shelf life of steaks of beef loins stored in 0.4% CO/60% CO_2/40% N_2 was 4 d longer than that of steaks stored in 70% O_2/30% CO_2 at 4°C[18]. Beef steaks that were exposed to pure CO before vacuum packaging had an extended shelf life compared with untreated controls. The total aerobic plate, lactic acid bacteria and psychrotropic counts of CO-treated steaks were 1–2 log cycles lower than those of controls after 8 weeks storage at 4°C[17]. In a study using pure bacterial cultures, the presence of CO at a concentration of 5–30% in air had no effect on the growth of *Pseudomonas aeruginosa*, inhibited the growth rate of *Escherichia coli* (in proportion to the concentration of CO), increased the lag phase of *Achromobacter* and inhibited the growth rate of *Pseudomonas fluorescens*[23].

Table 2. Estimate of carboxyhaemoglobin percent (COHb%) in human blood at different concentrations of carbon monoxide (CO) in the atmosphere, depending on the level of physical activity[a]

Exposure		COHb%		
CO concentration (mg/m³)	Time (h)	At rest	Moderate activity	Heavy work
10	8	1.3	1.4	1.4
25	1	1.0	1.5	2.0
40	1	1.3	2.2	2.9

[a]Data taken from Ref. 24

Toxicological aspects of CO

Health effects of CO

CO binds to the iron atom of haemoglobin in red blood cells, forming carboxyhaemoglobin (COHb). The affinity of haemoglobin for CO is ~240 times higher than its affinity for O_2. CO also binds to myoglobin, cytochromes and some enzymes, but these reactions are considered to be of less importance than the formation of COHb[2]. The binding of CO to haemoglobin is reversible, with a half-life of ~4.5 h in individuals who are at rest.

Although CO acts primarily by interfering with O_2 transport, it also reduces the delivery of O_2 to the various tissues[3]. In humans, health effects are mainly manifested in the cardiovascular system, the nervous system and in the foetus.

The COHb concentration in blood, often referred to as the COHb percent (COHb%), is a function of the CO concentration in the air, the exposure time, and the level of physical activity of the individual[24] (see Table 2). At a COHb concentration of ~2.5%, the most sensitive individuals (patients suffering from cardiovascular diseases) display changes in cardiac function and report chest pain. At somewhat higher COHb concentrations, they experience reduced working capacity and the onset of angina pectoris on exercise[25,26]. In healthy adults, no adverse health effects were described at CO concentrations that result in <5% COHb[27].

A small amount of CO is formed naturally in the human body, owing to the breakdown of haemoproteins.

Table 3. Association between different carboxyhaemoglobin (COHb) levels in blood and health effects[a]

COHb%	Observed health effects
≥50	Unconsciousness, lethal if not treated
≥30	Headache, nausea, vomiting, dizziness
≥10	Life threatening for heart and lung patients; headache in other individuals
≥5	Reduced maximum oxygen consumption during exercise in healthy individuals
≥5	Reduced visual perception, learning ability and fine motor performance
≥5	The foetus can be affected on carbon monoxide exposure of pregnant women
≥2.9	Angina patients endure less physical strain before experiencing attack
≥2.3	Reduced physical working capacity, especially endurance
≥2	Possible reduction in attention and ability to concentrate
≥2	Signs of local lack of oxygen and onset of chest pain in heart patients

[a]Data taken from Refs 25–27

Such production leads to a COHb concentration of ~0.5%. The average COHb concentration in non-smokers is 1.2–1.5%, and ~3–4% in smokers[27].

The absorption and excretion of CO from the body occur relatively slowly; thus, exposure to elevated CO levels over short time periods will not result in a significant increase in the COHb level in the blood. Table 3 details the various health effects observed at different COHb levels. This table confirms that exposure to CO that results in a COHb level greater than ~2% should be avoided to protect the most vulnerable individuals in the population.

In order to protect the most vulnerable in society, a Norwegian expert group on air pollution[27] recommended maximum CO concentrations for different exposure times that will prevent COHb levels from exceeding 1.5%, taking into consideration endogenous CO formation (Table 4).

Table 4. Estimates of carbon monoxide (CO) levels in ambient air that will result in carboxyhaemoglobin (COHb) levels of 1.5%, including endogenous CO production[a]

Exposure time	CO concentration in air (mg/m³)		
	At rest	Moderate activity	Heavy work
15 min	170	80	52
30 min	86	42	29
1 h	48	24	18
8 h	11.5	9.2	9.2

[a]Data taken from Ref. 27

Exposure to CO on consumption of fresh meat treated with a CO gas mixture

Very little information exists in the literature on the exposure to CO following the consumption of meat that has been treated with CO gas. The inhalation of air containing CO at a level of 57 mg/m³ (the acceptable level in working environments in the USA) would provide a COHb level for a prolonged time period (hours) of at least 14 times that of the level reached temporarily on the consumption of 225 g of meat that had been packaged in CO at the saturation level for myoglobin[14]. In this estimate, it was assumed that the saturation of the meat myoglobin and haemoglobin was maximal and that the transfer of CO from the gastrointestinal tract to the blood was 100%. Consequently, even for such a 'worst-case' scenario, the treatment of meat with CO gas appears to contribute very little to COHb levels, relative to levels that are considered safe in the working environment. The exposure of beef to an atmosphere containing 1% CO for 3 d resulted in ~30% saturation of the meat myoglobin[28]. CO is lost from previously CO-treated meat during storage in the absence of CO, with a half-life of ~3 d. When the beef was cooked at 195°C, only 0.1 mg of CO remained per kg of meat. The loss of CO amounted to ~85%.

Comparison of CO exposure from air and the consumption of gas-treated meat

Data are very scarce, but comparisons still allow crude estimates to be made. An adult inhales ~10–20 m³ of air in 24 h (depending on their level of activity). This is equivalent to 0.42–0.84 m³/h (or 3.36–6.72 m³ in 8 h). In order to prevent a maximum COHb level in the blood of 1.5% being exceeded, the CO concentration in air for a 1-h period of moderate physical activity should not exceed 24 mg/m³, or 9.2 mg/m³ in 8 h (according to Table 4). In contrast, the consumption of meat that had been treated for 3 d in an atmosphere containing 1% CO yielded ~0.1 mg of CO per kg of meat on storage and cooking[28]. Based on these data, a comparison can be made from the two methods of exposure to CO, and is shown in Table 5.

Equilibrium between CO present in the atmosphere and the COHb concentration in blood is reached only after a considerable period of time (depending on the concentration and level of physical activity). Even in a 'worst-case' scenario, equilibration between the CO concentration in the gastrointestinal tract and blood will take time. Furthermore, the absorption of CO from the gastrointestinal tract into the blood will in all probability be less effective than the absorption of CO from the lungs, which are composed of tissues that are designed to facilitate gas exchange between the alveoli and the blood. Consequently, it is highly probable that the consumption of one meal of CO-exposed meat per day will not result in measurable increases in the COHb level in blood.

Toxicological evaluation of the use of CO as a packaging gas for meat

Unfortunately, the European Union (EU) has not evaluated CO for use as a packaging gas for meat. However, CO_2 and nitrous oxide (N_2O) have both been approved for use for extraction purposes, and it was considered unnecessary to adopt an acceptable daily intake (ADI) value for these gases in this application[29].

In order to avoid possible adverse health effects in those individuals who are the most susceptible, a Norwegian expert group on air pollution recommended maximum CO concentrations in ambient air that result in COHb levels not exceeding 1.5% (including endogenous CO production)[27]. Estimates detailed above indicate that, even assuming an improbable 100% absorption of CO from the gastrointestinal tract into the blood, the consumption of meat that has been treated with 1% CO will result in COHb levels that are negligible (approximately three orders of magnitude lower) compared with those resulting from exposure in the working environment to CO at an acceptable level. Consequently, it is highly improbable that CO exposure from meat packaged in an atmosphere containing up to 0.5% will represent a toxic threat to consumers through the formation of COHb.

Alternatives to the MAP of retail meat

Currently, the most commonly used MA for the retail packaging of meat contains O_2 at a high concentration in combination with CO_2, such as ~70% O_2/30% CO_2. The shelf life of meat in a high O_2 atmosphere in commercial practice, typically at temperatures of 6–8°C, is ~7 d, being limited both by microbiological spoilage and discolouration. Meat that is stored in a high O_2 concentration is often spoiled by bacteria such as *Brochothrix thermosphacta* and pseudomonads[30]. In MAs with a high concentration of O_2, the meat normally maintains its bright red oxymyoglobin colour for 4–7 d before the colour starts deteriorating to grey–brown, owing to the formation of metmyoglobin[18]. This length of time is often not considered to be sufficient to display and sell the product.

The use of MAs with a high concentration of CO_2, either alone or in combination with up to 70% N_2, would increase the microbiological shelf life of the meat compared with that of meat in a MA with a high O_2 concentration. The absence of O_2 together with the presence

of CO_2 retards microbiological growth. Unfortunately, the colour of meat packaged in MAs containing CO_2 is less satisfactory, being either purple or grey–brown due to the formation of deoxymyoglobin or metmyoglobin, respectively. The meat inevitably discolours when the O_2 concentration is low. Metmyoglobin formation can be avoided by maintaining O_2 concentrations <0.01–0.1% for beef[31] and <0.5% for pork[32]. These low O_2 levels, particularly for beef, are difficult to achieve in most commercial packaging operations, because a small amount of air will unavoidably be incorporated in the MAs of the packages. MAs with a high CO_2 concentration seem to be useful for retail packaging if a low concentration of CO is also included to stabilize myoglobin and the meat colour.

Vacuum packaging is commonly used for the bulk storage, transportation and export of meat. However, vacuum packaging has not proved to be a successful method for the retail packaging of meat, because of the purple deoxymyoglobin colour of the meat and the visible exudate that occurs in the packages[5,30]. Meat that is packaged in a vacuum cannot be presented in the bright red oxymyoglobin state, which depends on the presence of a high concentration of O_2[30,33], or in the cherry red carboxymyoglobin state, which requires CO to be included in the MA.

One of the objections that has been raised against the use of CO as a packaging gas is the potential hazard it might represent to workers in meat plants. Although the use of pure CO for mixing in the plant would certainly pose such a risk, the delivery of 1% CO in a mixture with 99% N_2, which has been the practice of gas suppliers to the Norwegian meat industry, is recognized by the health authorities to be a very safe handling procedure.

MAs that contain 60–70% O_2 must be handled carefully, because they are explosive gas mixtures. Strict safety regulations apply to explosive gas mixtures, increasing the costs of equipment and packaging operations. The benefits of a CO mixture is that it carries no risk of explosion and therefore does not increase handling costs.

Despite the long-term knowledge of the many advantages of the use of CO as a component of MAs for meat, CO mixtures have not been adopted to any great extent by the global meat industry. In many countries, including the USA and countries within the EU, CO is presently not permitted for use in the MAP of meat[3,34]. However, Norwegian food control authorities have not opposed the use of CO as a packaging gas at concentrations of up to 0.5%. As a member of the European Economic Agreement, Norway is expected to adapt gradually to EU food regulations, including those relating to gases for the packaging of foods. The Norwegian meat industry is therefore preparing an inquiry, to be directed at the Norwegian and EU food control authorities, for the continued use of CO in the MAP of red meats, which will be partly based on the toxicological evaluation described in this article.

Table 5. Theoretical uptake of carbon monoxide (CO) in blood

Exposure method	CO intake in 1 h	CO intake in 8 h
Lungs (15 m³/d)	24 mg × 0.625 = 15.1 mg	9.2 mg × 5 = 46.0 mg
Meat (250 g, CO treated)	0.025 mg	0.025 mg

Consumers may evaluate the shelf life of packaged meat on the basis of its colour. A possible negative aspect of using CO in the MAP of retail meat is concern that consumers might misjudge the quality of a product, because its true microbiological status may be masked by its stable, cherry red carboxymyoglobin colour[1]. However, consumers will be able to detect spoilage by the presence of off-odours. At the current low concentrations, <0.5%, CO *per se* seems to have no or only minor effects on bacteria and the shelf life of the meat. The combination of CO with a high concentration of CO_2, for example 60–70%, is necessary for microbiological control. Although MAP enables centralized packaging operations with quality control to be carried out, MAP alone cannot guarantee the shelf life of the product. Sufficient shelf life can be obtained only through the proper quality control of raw materials, production, packaging, chill chain and retail conditions.

Conclusions

Gas mixtures that contain a low concentration of CO, up to 0.5%, and a high concentration of CO_2, ~70%, have many advantages with respect to shelf life, colour stability, labour safety and costs. The use of CO at such concentrations does not present any toxic threat to consumers. Considering the benefits the Norwegian meat industry has experienced with CO gas mixtures over the past decade, potential exists for their wider application in the retail packaging of meat.

Acknowledgement

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The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide

Oddvin Sørheim[a,*], Hilde Nissen[a], Truls Nesbakken[b]

[a]*MATFORSK-Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway*
[b]*Norwegian Meat Cooperative, Department of Research and Development, PO Box 360 Økern, N-0513 Oslo, Norway*

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Abstract

Ground beef, beef loin steaks and pork chops were packaged in modified atmospheres of 0.4% CO/60% CO_2/40% N_2 and 70% O_2/30% CO_2. In addition ground beef was packaged in clipped chub packs, beef loin steaks were vacuum packaged, and pork chops were packaged in an atmosphere of 60% CO_2/40% N_2 with each pack containing an O_2 absorber. The packs were stored in the dark at 4 or 8°C for up to 21 days. Meat in 0.4% CO/60% CO_2/40% N_2 had a stable bright red colour that lasted beyond the time of spoilage. The storage lives in this gas mixture at 4°C, as limited by off-odours, were 11, 14 and 21 days for ground beef, beef loin steaks and pork chops, respectively. The 70% O_2/30% CO_2 atmosphere resulted in an initially bright red to red colour of the meat, but the colour was unstable and off-odours developed rapidly. The off-odours probably were caused by *Brochothrix thermosphacta*, which grew in all meat types, or by pseudomonads in ground beef. Meat stored in chub packs, vacuum packs or 60% CO_2/40% N_2 with an O_2 absorber developed off-odours and microflora similar to those of meat in 0.4% CO/60% CO_2/40% N_2, but with less acceptable appearances. These results show that a low CO/high CO_2 atmosphere is effective for preserving retail-ready meat.

1. Introduction

The main reasons for modified atmosphere packaging (MAP) of red meats for retail sale are to prolong the microbiological shelf life and to maintain an attractive red colour of the product. Modified atmospheres (MA) usually consist of carbon dioxide (CO_2) for inhibiting microbiological growth, oxygen (O_2) for enhancing colour and, occasionally, nitrogen (N_2) as a filler. The most common gas mixture for retail-ready meat contains approximately 70% O_2 and 30% CO_2, and gives the product an extended shelf life compared to air (Gill, 1996). The shelf life and colour stability of meat stored in this gas mixture is still limited. To obtain a stable red colour for the meat, low concentrations (< 1%) of carbon monoxide (CO) can be introduced in the MA. Then, O_2 can be removed from the gas mixture and the concentration of bacteriostatic CO_2 can be increased. Anaerobic conditions extend the shelf life of meat considerably compared to air and O_2-enriched atmospheres (Gill & Molin, 1991). CO binds strongly to the meat pigment myoglobin to form stable carboxymyoglobin which has a cherry red colour (El-Badawi, Cain, Samuels, & Angelmeier, 1964). Low concentrations of CO have little effect on the microflora of meat (Clark, Lentz, & Roth, 1976; Gee & Brown, 1978; Luño, Beltrán, & Roncalés, 1998).

The Norwegian meat industry has for the past decade been using a gas mixture of approximately 0.3–0.5% CO, 60–70% CO_2 and 30–40% N_2 in retail-ready packages of beef, pork and lamb. Packages with this gas mixture now have a 50–60% share of the domestic, retail, red meat market. The technological, hygienic and toxicological aspects of using CO in MA for meat have recently been reviewed with the conclusion that CO used in concentrations up to 1% does not present a toxic hazard to the consumer (Sørheim, Aune, & Nesbakken, 1997a). However, CO may mask spoilage, because the stable cherry red colour can last beyond the microbiological shelf life of the meat (Kropf, 1980).

The inclusion of CO in MA for meat is controversial. CO is presently not allowed in MA for meat in the USA and in the EU (Cornforth, 1994; European Parliament and Council Directive, 1995). However, Norwegian food control authorities have up to now not opposed

* Corresponding author. Tel.: +47-64-970100; Fax: +47-64-970333; E-mail: oddvin.sorheim@matforsk.no

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the use of up to 0.5% CO in MA for meat. This would change with an adoption of EU food regulations in Norway. Consequently, the Norwegian meat industry is seeking amendments of current EU food regulations relating to the use of CO in MAP of red meats. If the use of CO should be disallowed, other means of maintaining the long shelf life and the attractive red colour of the meat will have to be sought.

The aim of the present experiments was to compare a commercial Norwegian $CO/CO_2/N_2$ mixture with alternative gas mixtures and packaging methods for their effects on the off-odour, microflora and colour of ground beef, beef loin steaks and pork chops stored at 4 or 8°C for up to 21 days.

2. Materials and methods

2.1. Preparation of meat

2.1.1. Ground beef

Twenty cow and bull carcasses of Norwegian Red Cattle, which weighed on average 275 kg, were electrically stimulated with 90 V and were chilled using programmed air temperatures between 12 and −5°C. Two days after slaughter the carcasses were deboned, and trimmings with 14% fat were ground through a 4 mm plate. The batch of ground beef was divided into 500 g portions.

2.1.2. Beef loin steaks

Loins (*m. longissimus lumborum et thoracis*) with ultimate pH values below 5.8 were deboned from 25 bull carcasses of Norwegian Red Cattle. These carcasses, which weighed on average 275 kg, were stimulated, chilled and deboned the same way as the carcasses used in the preparation of ground beef. The loins were vacuum packaged and aged for 11 days at 3°C. Thereafter, the loins were cut into steaks 2.5 cm thick, and were randomly assigned to retail packs which each contained two steaks.

2.1.3. Pork chops

Thirty pig carcasses of Norwegian Land Race, which weighed on average 75 kg, were blast-chilled. Four days after slaughter, bone-in loins were removed and crust-frozen in liquid N_2 at −50°C for 20 min to facilitate cutting of chops. The chops, which were 1.6 cm thick, were randomly assigned to retail packs which each contained two chops.

2.2. Packaging

Ground beef, beef loin steaks and pork chops were packaged in 0.4% CO/60% CO_2/40% N_2 (CO mixture) and 70% O_2/30% CO_2 (high O_2). In addition, ground beef was packaged in clipped chub packs, beef loin steaks were vacuum packaged and pork chops were packaged in 60% CO_2/40% N_2 with one Ageless® FX-100 O_2 absorber (Mitsubishi Gas Chem. Co. Inc., Tokyo, Japan) in each pack (mixture with O_2 absorber).

The meat was packaged at a commercial meat plant within 2 h of grinding or cutting. Meat in the CO mixture, the high O_2 mixture and the mixture with O_2 absorber was packaged in an Ilapak Delta 2000 flow-packaging machine (Ilapak Machine Auto S.A., Grancia, Switzerland). The CO mixture was a blend of 1% CO/99% N_2 with 100% CO_2. The high O_2 mixture was used as a preblend. The mixture with O_2 absorber was a blend of 100% N_2 with 100% CO_2 (all gases, Hydrogas, Porsgrunn, Norway). The initial gas volume to meat weight ratio in the packs was approximately 1.5 to 1. The packs consisted of polyethylene trays (Færch Plast, Holstebro, Denmark) wrapped in Cryovac BDF 550 shrinking film (Cryovac, Milan, Italy) with an O_2 transmission rate of 19 $cm^3/m^2/24$ h/atm at 23°C and 0% RH. Chub packs of ground beef were packaged in a clipping machine (Poly-Clip, Frankfurt, Germany) using a red, fishingnet-patterned, polyethylene film (SFK, Vidovre, Denmark) with an O_2 transmission rate of 500 $cm^3/m^2/24$ h/atm at 23°C and 0% RH. Beef loin steaks were vacuum packaged in a Multivac 5100 thermo-forming machine (Multivac, Wolfertschwenden, Germany) using a terephtalate/polyethylene upper film and polyamide/polyethylene lower film with O_2 transmission rates of 10 and 16 $cm^3/m^2/24$ h/atm at 23°C and 0% RH, respectively (Danisco, Horsens, Denmark).

2.3. Storage and sampling of meat

Five samples were collected from the ground beef batch, beef loins and pork loins before packaging, for pH measurements and microbiological analyses.

The packaged meat was stored in dark chilling rooms at 4 ± 0.5 or 8 ± 0.5°C for up to 21 days at least until off-odours developed. Five packs were removed per product, packaging method, storage temperature and sampling day after the following storage times:

- ground beef: 2, 4, 6, 8 or 11 days;
- beef loin steaks: 3, 7, 10 or 14 days; and
- pork chops: 3, 7, 10, 14, 17 or 21 days.

2.4. Gas analyses

The atmospheres of packs with MA were analysed for O_2 and CO_2 immediately after packaging (approximately every tenth pack) and at sampling (all packs). O_2 was determined using a Toray LC 700-F gas analyser (Toray Engineering, Osaka, Japan) and CO_2 using a Toray PG-100 gas analyser (Toray). The threshold levels for the O_2 and CO_2 analyses were 0.05 and 1%, respectively. Gas samples of 10 cm^3 were removed with a syringe through selfsealing patches on the packs.

2.5. pH

The pH measurements were made directly in the meat with an Ingold Xerolyt gel electrode (Mettler-Toledo A.G., Greifensee, Switzerland).

2.6. Odour

The meat was evaluated for odours by a three member trained panel between 0.5 and 1 min after opening of the packs. The off-odour scale used was: 1 = none, 3 = slight and 5 = extreme. Scores of 3 or below were considered acceptable.

2.7. Microbiology

Ten gram meat samples were collected from portions of the ground beef, and diluted in 90 g peptone water. A sample 25 cm^2 and 2–3 mm thick was removed from the surface of each beef loin or steak and pork loin or chop with a scalpel, and diluted in 100 ml peptone water. Each sample was macerated in a Stomacher for 1 min. Serial 10-fold dilutions of each Stomacher fluid were prepared, and 20 µl volumes of appropriate dilutions were plated in duplicate on the following media:

- plate count agar (PCA; Difco, Difco Laboratories, Detroit, MI, USA) for total viable counts;
- de Man, Sharpe and Rogosa agar (MRS; Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK) adjusted to pH 5.7 for lactic acid bacteria (de Man, Rogosa, & Sharpe, 1960);
- streptomycin thallous acetate actidione agar base (STAA; CM 881 with selective supplement SR 151; Oxoid) for *Brochothrix thermosphacta*;
- pseudomonads agar base (CFC; CM 559 with selective supplement SR 103; Oxoid) for pseudomonads;

In addition, 1 ml portions of appropriate dilutions were plated in duplicate on petrifilm coliform count plates (3M Microbiology Products, St. Paul, MN, USA) for enumeration of coliforms and *Escherichia coli*.

Plates of PCA, MRS, STAA and CFC were incubated at 20°C for four days, and petrifilm plates at 30°C for up to 2 days, all aerobically. Counts were expressed as colony forming units (CFU) per g or cm^2.

2.8. Colour

A six-member trained panel evaluated the colour of the meat in intact packs under 1200 ± 200 lux Warmton Lumilux L36W/31 yellow–white light (Osram, Drammen, Norway). The colour was assessed on a scale where 1 = bright red (ground beef and beef loin steaks) or light bright red (pork chops), 2 = red (ground beef and beef loin steaks) or light red (pork chops), 3 = slightly brown, grey or green, 4 = moderately brown, grey or green and 5 = extremely brown, grey or green (National Live Stock and Meat Board, 1991).

A Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) with 8 mm viewing port and illuminant D_{65} was used for measuring CIE a^* values (redness). The colour was measured directly at the meat surface within 1 min of opening of each pack.

Ground beef in chub packs was not included in the colour analyses because the red packaging film hides the colour of the product. With pork chops, the colour of only the *m. longissimus lumborum et thoracis* was analysed.

2.9. Statistics

Analysis of variance by Tukey's multiple comparisons test was performed using the Systat programme, version 6 (Systat Inc., Evanston, IL, USA).

3. Results

3.1. Gas composition

The initial O_2 concentrations in packs with the CO mixture and the mixture with O_2 absorber were all below 0.5% immediately after packaging. O_2 was not detected in these packs after 2 or 3 days storage. The level of O_2 in packs of high O_2 was reduced from the initial 70 to 60–65% during storage for up to 21 days. Concentrations of CO_2 in the packs were generally reduced by one fifth after 2 or 3 days storage, and were then stable (data not shown).

3.2. Storage life of ground beef

The time to develop off-odours was 2 to 3 days longer for ground beef stored in the CO mixture and in chub packs than in high O_2, and it was 4 or 5 days longer at 4 than at 8°C for all three packaging methods (Table 1). In high O_2, the total viable counts increased faster and were higher ($p < 0.01$) than for the other two types of packaging after 2 days at either 4 or 8°C [Fig. 1(a)]. The total viable counts were more than 90% lactic acid bacteria (data not shown). The high numbers of lactic acid bacteria in ground beef, up to approximately $\log_{10}$ 8 CFU/g, caused a decrease in the pH value from the initial 5.7 to 5.2 after 6 days when the meat was stored in the CO mixture or chub packs at 8°C (data not shown). At 4°C, the pH value was reduced to 5.5 after 11 days in both those packaging systems. The numbers of *B. thermosphacta* increased, in meat in high O_2 [Fig. 1(b)]. In meat in high O_2 the numbers of pseudomonads increased up to approximately $\log_{10}$ 7 CFU/g, but only to $\log_{10}$ 5 and 6 CFU/g in

meat in the CO mixture or chub packs, respectively (data not shown).

Ground beef in the CO mixture had a stable bright red colour, as shown by both the low colour scores and the high a^* values [Fig. 1(c) and (d)]. Meat in high O_2 was significantly less red ($p < 0.05$) than meat in the CO mixture, with higher colour scores and lower a^* values at day 2 and at later storage times at both 4 and 8°C. The colour of meat in high O_2 deteriorated with time, significantly faster ($p < 0.01$) at 8 than at 4°C.

Table 1
Time for development of off-odours in different types of meat in various packagings at storage temperatures of 4 or 8°C

Product	Packaging[a]	Time of off-odour detection (days)	
		4°C	8°C
Ground beef	CO mixture	11	6
	High O_2	8	4
	Chub packs	11	6
Beef loin steaks	CO mixture	14	7
	High O_2	10	7
	Vacuum packs	14	7
Pork chops	CO mixture	21	14
	High O_2	14	7
	Mixture with O_2 absorber	17	10

[a] CO mixture = modified atmosphere of 0.4% CO/60% CO_2/40% N_2; High O_2 = modified atmosphere of 70% O_2/30% CO_2; Mixture with O_2 absorber = modified atmosphere of 60% CO_2/40% N_2 with an O_2 absorber in the pack.

3.3. Storage life of beef loin steaks

At 4°C, off-odours developed 4 days later in beef loin steaks in the CO mixture and in vacuum packs than in high O_2 (Table 1). At 8°C, no differences in the development of off-odours were observed. Off-odours developed 4 to 7 days earlier in meat at 8 than at 4°C. The type of packaging did not significantly affect ($p < 0.05$) the total viable counts on the meat, but the counts were significantly higher ($p < 0.01$) at 8 than at 4°C after both 3 and 7 days of storage [Fig. 2(a)]. The numbers of *B. thermosphacta* were less than $\log_{10}$ 4 CFU/cm^2 in meat in all types of packaging at all times, but were significantly higher ($p < 0.05$) on meat in high O_2 at 7 and 10 days than on meat in the CO mixture and in vacuum packs at equivalent times [Fig. 2(b)]. The numbers of pseudomonads did not exceed $\log_{10}$ 3.5 CFU/cm^2 at any sampling time, and were not significantly affected ($p > 0.05$) by the type of packaging or the storage temperature.

The colour of the beef loin steaks in the CO mixture was stable bright red throughout storage at both 4 and 8°C, as shown by the low colour scores and high a^* values [Fig. 2(c) and (d)]. Steaks in high O_2 were also bright red with high a^* values at day 3, but these steaks discoloured gradually between days 3 and 10, significantly faster ($p < 0.05$) at 8 than at 4°C. Meat in vacuum packs was slightly discoloured with low a^* values throughout storage. The colour scores and a^* values of vacuum packaged steaks were not significantly affected ($p > 0.05$) by the storage temperature.

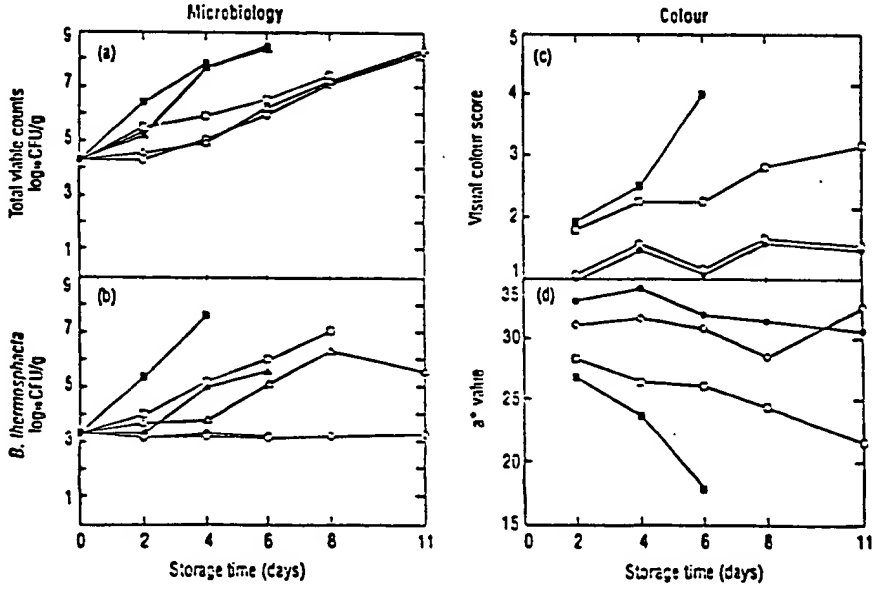

Fig. 1. Mean values ($n = 5$) for (a) total viable counts, (b) numbers of *Brochothrix thermosphacta*, (c) visual colour scores and (d) CIE a^* values for ground beef stored in 0.4% CO/60% CO_2/40% N_2 at 4°C (○) or 8° C (●), in 70% O_2/30% CO_2 at 4°C (□) or 8°C (■), or in chub packs at 4°C (△) or 8°C (▲). Colour was assessed on a scale where 1 = bright red and 5 = extremely discoloured.

3.4. Storage life of pork chops

For pork chops, off-odours developed more slowly in meat in the CO mixture than in meat in the mixture with O_2 absorbers or in high O_2 (Table 1). Off-odours were detected 7 days earlier at 8 than at 4°C for chops in each type of packaging. The type of packaging did not affect the total viable counts on the pork chops [Fig. 3(a)]. However, the counts were greater on meat stored at 8 than at 4°C. The numbers of *B. thermosphacta* on chops in high O_2 were significantly higher ($p < 0.01$) than on chops in the CO mixture or in the mixture with O_2 absorbers after 7 days at 8°C or 10 days at 4°C, and reached approximately $\log_{10}$ 6 CFU/cm^2 [Fig. 3(b)]. The numbers of pseudomonads did not exceed $\log_{10}$ 3 CFU/cm^2 on any of the pork chops.

The colour of pork chops in the CO mixture was light bright red with high a^* values throughout storage [Fig. 3(c) and (d)]. Chops in high O_2 were red at day 3, but discoloured during storage, significantly faster ($p < 0.05$) at

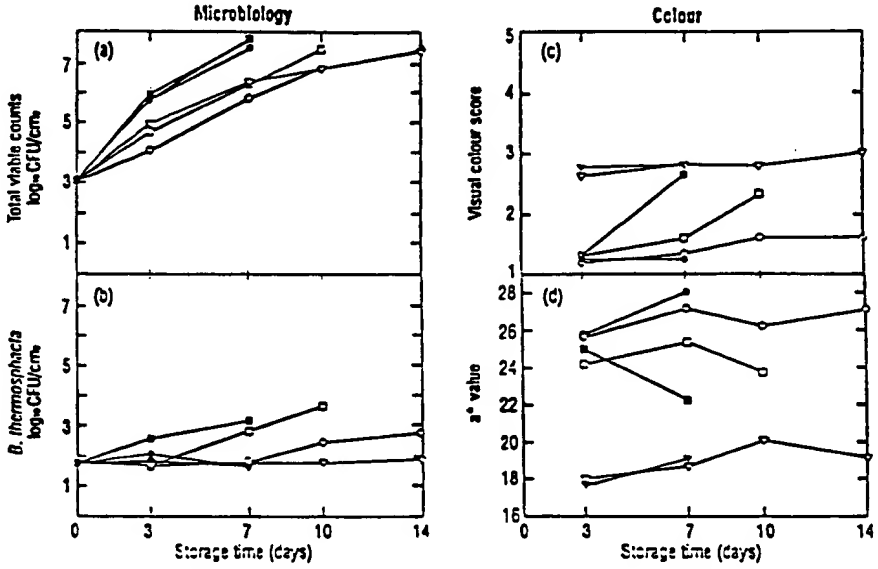

Fig. 2. Mean values ($n = 5$) for (a) total viable counts, (b) numbers of *Brochothrix thermosphacta*, (c) visual colour scores and (d) CIE a^* values for beef loin steaks stored in 0.4% CO/60% CO_2/40% N_2 at 4°C (○) or 8°C (●), in 70% O_2/30% CO_2 at 4°C (□) or 8°C (■), or in vacuum packs at 4°C (▽) or 8°C (▼). Colour was assessed on a scale where 1 = bright red and 5 = extremely discoloured.

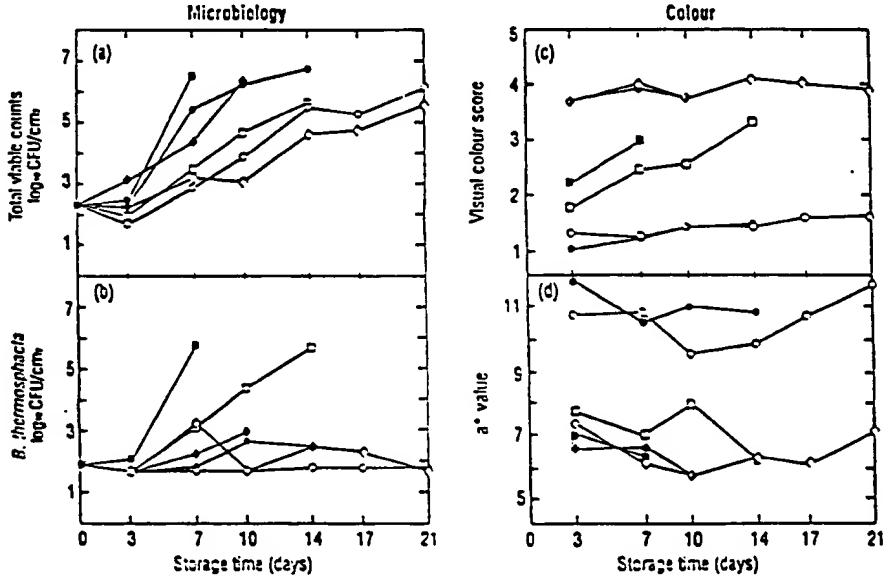

Fig. 3. Mean values ($n = 5$) for (a) total viable counts, (b) numbers of *Brochothrix thermosphacta*, (c) visual colour scores and (d) CIE a^* values for pork chops stored in 0.4% CO/60% CO_2/40% N_2 at 4°C (○) or 8° C (●), in 70% O_2/30% CO_2 at 4°C (□) or 8°C (■), or in 60% CO_2/40% N_2 with O_2 absorbers at 4°C (◇) or 8°C (◆). Colour was assessed on a scale where 1 = light bright red and 5 = extremely discoloured.

8 than at 4°C. Approximately 75% of the chops in high O_2 had black back bones at the time of sampling. Chops in the mixture with O_2 absorbers were moderately discoloured from day 3 to the end of storage. These chops had a^* values similar to those of chops in high O_2.

4. Discussion

4.1. Off-odour and microflora

The shelf life of the meat, as determined by the time to develop off-odours, was influenced by the packaging method, the storage temperature and the initial microbiological load on the meat. Storage of meat in the CO mixture, in vacuum packs or in chub packs gave the longest shelf lives. Meat stored in high O_2 generally developed off-odours 2–7 days earlier at 4 or 8°C than meat packaged in the other gas mixtures or by the other methods.

The differences in the rates of development of off-odours, as affected by the packaging method, were seldom related to any differences in numbers of total viable counts. However, the development of off-odours from the three meat types, especially ground beef and pork chops in high O_2, coincided with the attainment of high numbers of *B. thermosphacta*. For ground beef, storage in the CO mixture retarded growth of *B. thermosphacta* even more than storage in chub packs. At chill temperatures above 1°C, *B. thermosphacta* often causes spoilage of meat stored in high O_2 atmospheres (Dainty & Mackey, 1992). High concentrations of CO_2, removal of O_2 and low storage temperature inhibit the growth of *B. thermosphacta* (Gill, 1996; Nissen, Sørheim, & Dainty, 1996). Pseudomonads probably contributed to the off-odours of ground beef. Meat in high O_2 is often spoiled by *Pseudomonas* spp., but the growth of pseudomonads is retarded under anaerobic conditions (Dainty & Mackey, 1992; Gill, 1996). A shift in the metabolism of lactic acid bacteria under aerobic conditions can also produce off-odours (Nissen et al., 1996). In the present experiments, the numbers of coliforms or *E. coli* did not exceed $\log_{10}$ 3 CFU/g or cm^2 in any samples. Therefore, those organisms probably did not contribute to off-odours.

For pork chops, the effect of CO on the microflora can be evaluated because the gas compositions of the CO mixture and of the mixture with O_2 absorber were identical, except for the inclusion of 0.4% CO in the former. Although a 4 day increase in the time to develop off-odours was observed with the CO mixture, there was no significant reduction in the microbiological counts. Luño et al. (1998) used 1% CO in high O_2 atmospheres and noted a delay in the onset of off-odours without any reduction in the numbers of psychrotrophic bacteria. However, Clark et al. (1976) found that the addition of 0.5-10% CO to N_2 atmospheres reduced the number of psychrotrophic bacteria and increased the odour shelf life of beef. For example, 1.0% CO in 99% N_2 increased the time to develop off-odours at 5°C from 18 to 24 days. The lack of such an effect of CO on bacteria in our experiments may be due to the use of 60% CO_2 overshadowing any effect of CO.

The use of CO makes it possible to dispense with O_2 and so to increase the CO_2 concentration in a MA to about 60%. Our data suggest that 0.4% CO probably has little or no direct effect on the growth of bacteria. Other studies have shown that increasing the CO_2 concentration from 20 to 100% increases the bacteriostatic effect of the gas, but the efficiency is highly dependent on low storage temperatures (Gill & Molin, 1991; Nissen et al., 1996). The high CO_2 concentration and absence of O_2 in the CO mixture will favour the growth of lactic acid bacteria, which usually cause a mild form of spoilage only late in the development of the spoilage flora (Gill, 1996).

The present experiments were performed at acceptable and abusive storage temperatures to assess the effects of temperatures commonly encountered in the distribution and sale of retail-ready meat. The storage temperature strongly affected the rates of growth of microflora and the time to develop off-odours. Consequently, independently of the packaging method, the shelf life of meat can be considerably extended by maintaining low temperatures in the chill chain (Gill & Molin, 1991; Nissen et al., 1996).

4.2. Colour

The CO mixture gave a stable bright or light bright red colour with consistent high a^* values for all three products, irrespective of the storage temperature. The initial level of residual O_2, up to 0.5%, did not adversely affect the visual scores and instrumental values for the colour of meat stored in the CO mixture.

CO binds to myoglobin and forms cherry red carboxymyoglobin (El-Badawi et al., 1964). This pigment is spectrally similar to the bright red oxymyoglobin which normally develops at the surface of fresh meat in air. Carboxymyoglobin is less readily oxidized to brown metmyoglobin than is oxymyoglobin, because of the strong binding of CO to the iron-porphyrin site on the myoglobin molecule (Lanier, Carpenter, Toledo, & Reagan, 1978; Wolfe, 1980). Consequently, CO in concentrations of 0.5–2.0% enhances and stabilizes a bright red colour of meat (Kropf, 1980; Sørheim et al., 1997a). In a recent study, 1% CO in combination with 24 or 70% O_2 stabilized the colour of beef by reduced formation of metmyoglobin after storage at 1°C for up to 29 days (Luño et al., 1998). However, in a study of beef stored in a MA of 2% CO/78% CO_2/20% N_2, the colour of the meat was characterized as "too artificial" by

a sensory panel (Renerre & Labadie, 1993). From our studies and experience from the Norwegian meat industry, 0.4% CO seems sufficient to produce a stable, attractive, bright red colour of meat.

All three meat types stored in high O_2 were bright red to red with high a^* values early in the storage periods, approaching the colour of meat in the CO mixture. As the microbiological counts of meat in high O_2 increased, the colour deteriorated, faster at 8 than at 4°C. Meat stored in a MA of high O_2 develops a thicker layer of oxymyoglobin than meat stored in air (Renerre & Labadie, 1993). However, the oxymyoglobin gradually oxidizes to metmyoglobin, and the oxidation is faster at higher temperatures.

For cut bone, haemoglobin released from disrupted red blood cells in the marrow will accumulate at the surface and ultimately become black after the bone has been exposed to air or O_2 (Gill, 1996). Although bone blackening was not considered in the present visual colour evaluation, it can negatively affect the saleability of bone-in meat at retail display. The cut bones of pork chops stored in high O_2 blackened during storage, but this discoloration was not observed on bones in the CO mixture and the mixture with O_2 absorbers.

Beef loin steaks stored in vacuum packs were slightly discoloured with low a^* values at both 4 and 8°C. In these packs, meat juices were observed between the upper and lower films, but that did not influence the colour evaluations.

O_2 absorbers in packs with high CO_2 facilitate the removal of residual O_2 and maintain atmospheres free of O_2 during storage (Smith, Abe, & Hoshino, 1995). Low levels of residual O_2, above 0.01–0.15% for beef and 0.5–1.0% for pork, will inevitably discolour the meat (Penney & Bell, 1993; Gill, 1996; Sørheim et al., 1997b). When no CO is present in an O_2 depleted MA, it is essential to remove the residual O_2 as fast and completely as possible to avoid formation of metmyoglobin. In these experiments, pork chops stored in the gas mixture with O_2 absorbers were moderately discoloured during the whole storage period at 4 or 8°C. Despite the obvious visible differences, these chops had similar c^* values to the chops in high O_2. The discoloured surface made the chops unfit for sale, even in the early stage of storage. The present findings contrast with previous results, where the colour of porcine *m. longissimus thoracis et lumborum* was significantly improved by using O_2 absorbers in MAs of CO_2 with residual O_2 (Sørheim et al., 1997b). The present discoloration could be caused by incomplete use or function of the absorbers (Gill, 1996).

4.3. Benefits and disadvantages of a MA with low CO/high CO_2

An objection raised against using CO as a small component of a MA for retail-ready meat is the possibility that the colour stability can exceed the microbiological shelf life, with the risk of masking spoilage of the meat (Kropf, 1980). Therefore, the consumer must evaluate the microbiological condition of meat in a CO mixture by off-odours. When a MA with CO is applied commercially, it is important to have a proper control of the hygienic condition of the meat raw materials and the chill chain temperatures.

CO used in concentrations below 1.0% does not present any hazard to the consumer, because consumption of meat packaged in such concentrations of CO will result in only negligible levels of carboxyhaemoglobin in the blood of consumers (Sørheim et al., 1997a). By delivering CO in a 1% mixture with 99% N_2, which is the practice of Norwegian gas suppliers, CO is considered safe for use in the working environment. Other MAs with high levels of O_2, up to 70%, must be regarded as explosive gas mixtures, which must be used with appropriate precautions for safety (Luño et al., 1998).

The suitability of gas mixtures and packaging methods for red meats for retail display depends on their ability to both reduce spoilage and stabilize colour. Gas mixtures with low concentrations of CO and high concentrations of CO_2 provide a combination of a long microbiological shelf life and a stable, bright red colour of meat. Meat packaged in a MA with high O_2 can achieve an initial bright red colour, but the microbiological shelf life and the colour stability are both considerably lower than those of meat in the CO mixture. Using CO eliminates the need to have O_2 as a component of the MA. Other MAs and packaging methods, like high CO_2 with O_2 absorbers, chub packs and vacuum packs may give a shelf life comparable to that of the CO mixture, but with a less acceptable colour or appearance of the meat. Thus, there appears at present to be no fully satisfactory alternative to the CO mixture used in packaging of retail-ready red meats in Norway.

Acknowledgements

The financial support of this study from the Research Council of Norway is highly appreciated. Vestfold-Buskerud Slakteri A/L, Sem and Hydrogas AS Utviklingssenter, Porsgrunn, are greatly thanked for packaging of the meat. We appreciate the gift of Ageless® O_2 absorbers from Cryovac Europe, Norderstedt, Germany. The technical staff and Per Lea (statistics) at MATFORSK are thanked for their skilful assistance in the study.

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[logo]

SWEDISH MEATS

Date	Ref. No.
April 9, 1999	33

Reference:
Lars Wedén, (+46) 8-725 81 03

Date	Ref. No.

Svein E. Skorstad
Norsk Kjott [Norwegian Meat]
P. O. Box 60 Refstad
N-0513 Oslo 5 NORWAY

RECEIVED:	*April 14, 1999*
CASE WORKER:	*K. Framstad*
FILE NO.:	*561*
J. NO./DOC. NO.:	*99/00721*

Brother:

Please refer to our conversation at the Scandinavian Butchers' Association meeting on March 19 regarding CO as a packaging gas. Here at Swedish Meats we are committed to creating opportunities to increase the meat packaging done by the manufacturer in Sweden. An approval of CO as a gas for use in foods would entail significantly greater possibilities of brand profiling of meat products in the future, which could help improve our service to the retailers. Moreover, efficient factory packaging could even reduce costs across the entire distribution chain ending with the consumer.

We therefore support Norsk Kjott's proposal to submit a joint application to the EU Commission for the addition of CO to EU's list of additives. The plan is to submit a joint application in June 1999.

Sincerely,

Swedish Meats

[signature]
Lars Wedén

[letterhead information]

DANSKE SLAGTERIER [Danish Bacon and Meat Council]
Axelborg, Axeltorv 3
1609 Copenhagen V
Denmark
Tel. (+45) 33 11 60 50
Fax (+45) 33 11 68 14
Telex 22 975 ds dk
Telegram "danslagt"
[Logo]

February 11, 2000
Tel. (+45) 33 73 25 68

Norsk Kjott [Norwegian Meat]
att: Jorunn Vormeland
PB 360 Økern
N-0513 Oslo
Norway

[Stamp:]Received: *February 14, 2000*
Case Worker: *JVO*
File No.: *562*
J. No./Doc. No. *00/00204*

Use of CO as Packaging Gas

In Norway, CO has so far been used in very minute quantities together with other packaging gases for gas packaged cuts of meat and ground meat. This packaging gas has not been approved in the EU yet, and has not been used in Denmark this far.

CO and the other packaging gases help ensure the storage life and color stability of fresh meat, which are important for central packaging and distribution of meat. The trend in Denmark is toward increased central packaging of fresh meat, since this is both efficient and ensures high microbiological quality. It would therefore be interesting to take advantage of the positive Norwegian experiences with the use of CO in this country, as well.

Published research shows that the risk of growth of a range of pathogenic bacteria is the same or reduced when using CO in combination with the traditional packaging gases. The use of this gas can thus help improve food safety (Food Microbiology and Food Safety into the Next Millennium, Proceedings of the Seventeenth International Conference of International Committee on Food Microbiology and Hygiene, Netherlands, 1999).

The use of CO in the given concentration of 0.3–0.5% should not represent any toxicological risk to consumers, CO is generally supplied in a concentration of 1% in a mixture with either N_2 or CO_2 and does therefore not represent any workplace hazard to operators during the packaging process.

We are not aware that the use of CO was discussed in the process of drafting Directive 95/2/EU of February 20, 1995 concerning additives other than colorings and sweeteners. This may be due to the fact that no country had expressed any interest in using this kind of gas at the time.

Since there are advantages to the use of CO as a packaging gas, as mentioned, and since there are no negative effects to either consumers or packaging operators, DANSKE SLAGTERIER can support an

application to the EU Commission to include CO on the list of approved additives, possibly limiting the amount.

Sincerely,

DANSKE SLAGTERIER
[Signature]
Anne Birgitte Lundholt
(Managing Director)

(10)

Asociación de Industrias de la Carne de España

TELEFAX

To: Dirk Dobbelaere
Secretary (CLITRAVI)

Subject: CO gas at meat packaging gas

Date: 14 of February 2000

Nº of pages: 1

Dear Dirk:

After reading the scientific documents that Mr. Truls Nesbakken handed out in the last T&L working group meeting. We will support the Norwegian proposal for authorization CO gas as a packaging gas within the UE.

We look forward to hearing from you soon.

Yours sincerely,

Miryam de Miguel
Dpto. Calidad-AICE

For info
To Norwegian CLITRAVI member
From CLITRAVI

General Rodrigo, 6 • 28003 MADRID
Teléfono: 91 554.70.45 - Fax: 91 554.78.49 - E-mail: aice@aice.es

Finnish Meat Research In. ut

(11)

21.2.2000

Truls Nesbakken
Fagsentret för kjøtt
PB 396 Økern
0513 Oslo
Norge

Use of CO as packaging gas for meat and meat products

After going through the scientific documents sent to us and having own projects supporting the results, our institute is ready to support the Norwegian proposal to allow CO gas as a packaging gas in EU.

Finnish Meat Research Institute

Markku Raevuori
Managing Director

Raymond Tuominen
Laboratory Director

Finnish Meat Research Institute
P.O. Box 56
FIN-13101 Hämeenlinna

Address
Luukkaankatu 8
FIN-13110 Hämeenlinna

Telephone
+358 3 570 531

Telefax
+358 3 5705 499

000147

The Use of CO as a Packaging Gas for Fresh Meat.

By Magne Yndestad

A previous report on the use of CO as a packaging gas concluded that there is unsatisfactory documentation on factors such as the development of pathogenic bacteria in the gas mixture in question (0.4% CO/60% CO_2/40% N_2). The Norwegian Research Center for Meat forwarded recent and complementary documentation on September 13, 1999.

Following scientific review, the content of this documentation can be summed up as follows:

Bacteriological Conditions

Numerous studies have been undertaken regarding trial storage involving concentrations of CO_2 in a range consistent with the "Norwegian" mixture (60-75% CO_2). Moreover, there are articles documenting the bactericidal effect of various other concentrations of gas mixtures containing CO_2. The conclusion to these trials is the following:

The low CO concentration (≤0.5 CO) has no apparent effect on bacterial flora in products packaged with gas. This also holds true for N_2 (filler gas).

Concentrations of CO_2 below 5% may stimulate the growth of certain types of bacteria. Between 5 and 50%, we see an approximately linear inhibiting effect. This effect is somewhat significant, since the inhibition of growth of the sensitive flora is as much as 50% at 10% CO_2. The documented effect of CO_2 in high concentrations primarily applies to the psychrotrophic flora, including the most important spoilage bacteria.

As for the pathogenic bacteria, scientific literature in general points to the same tendency, i.e. inhibition of growth at both 4°C and higher temperatures (e.g. 10° C).

In comparison to other packaging methods or gas mixtures used, the mixture in question seems favorable both in terms of storage life and in terms of the relevant pathogens.

Following the last round of applications, The Norwegian Research Center for Meat has performed a relatively extensive study on freshly ground meat packaged in 0.3-0.5% CO/60-70% CO_2 and 30-40% N_2. Various pathogens, such as *E.coli* O157:H7, *Listeria monocytogenes*, and *Yersinia enterocolitica* were tested in this trial. The Research Center has evaluated factors such as the important possibility that the strong suppression of the general psychotrophic flora may favor certain pathogens, which will not be inhibited to the same degree. The main conclusion, however, is that the aforementioned pathogens are inhibited both at 4°C and 10°C. Comparing the CO packaging method to packaging employing a high concentration of O_2 or vacuum, shows that the risk for growth of the applied pathogens is identical or lower when packaging with CO.

The Research Center has studied the circumstances concerning salmonella bacteria and the gas mixture in question in the same products. Since none of the cultures grew at +4°C, studies were only undertaken at 10°C.

In this case, storage with packaging gas containing CO performed worst with regard to *S. dublin*, *S. enteritidis* and *S.diarizonae*, as a relatively strong growth occurred following Day 2. *S. typhimurium* too had considerable growth, although "sausage" packaging scored lower.

This is completely in line with what is known about a whole range of salmonella bacteria in foods, i.e. that they hold up very well when competing with other bacteria, and also grow very well at temperatures around 8-10°C.
These facts emphasize the importance of cooling regardless of what packaging method is chosen.

Sensorial Circumstances

The last report pointed to the particular fact that the CO packaged meat could retain a fresh red color for days after spoilage set in. Hence, the consumer cannot *see* whether the meat he or she buys is spoiled, as opposed to fresh meat packaged in other types of gas packaging.
The Research Center notes that when opening a package, the consumer will detect any spoilage odor, and hence not eat the product. This may be true, but it is a fact that many people won't react to any incipient decay when the products looks completely "fresh." However, the packaging method for which approval is sought is meant for fresh meat that will be treated with heat prior to use. This is an additional safety factor that is important in a comprehensive evaluation.

Conclusion

The first bacteriological/sanitary statement made was based on the documentation available at the time. The new data and other relevant information from scientific literature indicate that there is sufficient evidence that the use of CO as a packaging gas as described in the application won't result in any increased risk of transmittal of food-borne diseases among consumers.

[Handwritten:]
From the report "Fresh Meat in Consumer Packaging" with modified gas containing CO_2 [illegible]

IV. Report by Tore Aune: "Fresh Meat in Consumer Packaging – A Toxicological Evaluation of the Use of up to 0.5% CO in a Gas Mixture."

FRESH MEAT IN CONSUMER PACKAGING – A TOXICOLOGICAL EVALUATION OF THE USE OF UP TO 0.5% CO IN A GAS MIXTURE

By Tore Aune

Carbon monoxide (CO) is a colorless gas that is primarily generated by incomplete combustion of organic material. The background concentration of CO in the atmosphere is approximately 0.01-0.09mg/m^3 (0.009-0.08 ppm), while the concentration in larger cities may excede 50mg/m^3 as an 8 hour mean, depending on traffic.

General Health Effects

CO attaches to the iron of the hemoglobin in the red blood cells during generation of carboxyhemoglobin (COHb), and can thus affect the transport of oxygen in the blood and the supply of oxygen to the tissues. Compared to its affinity to oxygen, hemoglobin has approximately 240 times greater affinity to CO. CO also attaches to myoglobin, cytochromes, and some other enzymes, but these reactions are considered less important than the formation of carboxyhemoglobin (WHO 1979). The health impact on humans is mainly restricted to effects on the cardiovascular system, the nervous system, and certain types of proteins and cells in the bloodstream, as well as effects on embryos (SFT 1992).

The carboxyhemoglobin percentage (COHb %) is a function of the CO concentration in the inhaled air, the exposure time and the level of physical activity (Coburn et al., 1965) (see Table 1). A CO exposure resulting in a COHb concentration above 2% in the bloodstream of the most sensitive individuals (cardiovascular patients) has been shown to give symptoms of localized oxygen deficit and chest pains. Reduced work capacity occurs at a somewhat higher COHb%, and persons suffering from angina can tolerate less strain before an attack occurs. No health effects have been detected in healthy adults at COHb concentrations below 5%.

Table 1: Blood carboxyhemoglobin percentage as a function of CO concentration in air, exposure time and different degrees of physical activity (Coburn et al., 1965):

Exposure		COHb%		
CO Conc.	Time in Hours:	At rest	Moderate Activity	Strenuous Activity
10 mg/m^3	8	1.3	1.4	1.4
25 mg/m^3	1	1.0	1.5	2.0
40 mg/m^3	1	1.3	2.2	2.9

CO attachment to the hemoglobin is reversible. The half-life at ventilation at rest is approximately 4.5 hours.

A small amount of CO is continually formed in the body as a result of the decomposition of substances such as hemoproteins. This results in a COHb% of approximately 0.5. The uptake of CO through inhalation comes in addition to that. The average COHb level in non-smokers is estimated at 1.2-1.5%, while the level is 3-4% in smokers.

Survey of Health Effects Associated with CO Exposure

The negative health effects of CO are due to the fact that CO competes with oxygen for points of attachments on the hemoglobin molecule. Moreover, the release of oxygen in the tissues is reduced (WGHO 1987). Myoglobin is closely related to hemoglobin. It stores oxygen and promotes the diffusion of oxygen to muscle cells. In cardiac and skeletal muscles, myoglobin binds CO with an affinity that is 30-50 times higher than the corresponding affinity for oxygen. No reported studies have shown that the binding of CO to myoglobin can cause any health effect at a COHb level of 4-5%.

Uptake and liberation of CO occur at a relatively slow pace (hours), which means that short-time exposure to elevated CO levels will not result in any noticeable increase in the COHb level. SFT report No. 92/16 (1992) includes an overview of the correlation between blood COHb levels and health effects (Table 2).

Table 2: Correlation between blood carboxyhemoglobin levels and health effects (SFT 1992):

COHb%	Observed Effects in Humans:
50 and above	Unconsciousness, lethal when untreated.
30 and above	Headache, dizziness, nausea, and vomiting.
10 and above	May be lethal to cardiovascular patients. Headache in healthy individuals.
5 and above	Reduction of peak oxygen consumption under strenuous activity in healthy individuals.
5 and above	Impaired vision, learning ability and fine motor response.
5 and above	Exposure during pregnancy may affect the embryo.
2.9 and above	Individuals suffering from angina can tolerate less strain before an attack occurs.
2.3 and above	Reduced capacity for physical work, especially stamina.
2 and above	Possible reduced ability to concentrate and pay attention.
2 and above	Symptoms of localized oxygen deficit and incipient chest pains in cardiac patients.

The literature in the field does not seem to indicate that health effects have been proven in healthy adults exposed to CO resulting in a blood COHb concentration of less than 5%.

However, the data indicate that a COHb level of 2-3% may have negative effects on sick and sensitive individuals, such as people suffering from cardiovascular diseases.

Exposure to CO through the Air

With regard to CO as an air pollution factor, a team of Norwegian experts (SFT 1992) suggested air quality criteria at CO concentrations resulting in a maximum of 1.5% COHb during light physical activity (including the CO produced endogenically). The correlation between CO concentration, activity level, and exposure time in order not to exceed 1.5% blood COHb is shown in Table 3.

Table 3: Calculation of CO concentrations in the air resulting in a COHb level of 1.5%, including endogenic CO production (SFT 1992):

	CO Concentration, mg/m^3		
Exposure Time:	At Rest:	Moderate Physical Activity:	Strenuous Physical Activity:
15 min	170	80	52
30 min	86	42	29
1 hour	48	24	18
8 hours	11.5	9.2	9.2

Exposure to CO through Consumption of Fresh Meat Treated with a Gas Mixture

There is a paucity of information in scientific literature concerning exposure to CO through the consumption of fresh meat treated with a gas mixture containing CO. One of the most interesting references in this regard is a 1954 publication by A. L. Tappel et al., which is unfortunately not easily accessible. However, their work has been cited in other publications, e.g. in the study by Clark et al. (1976): Tappel et al. considered a US industrial sanitary norm for CO of 50 ppm (8 hours/day), and found that such exposure would result in a blood COHb level over a longer period of time that is approximately 14 times higher than the temporary increase caused by consumption of approximately 225 g meat, provided that the myoglobin and hemoglobin in the meat are saturated with CO, and that 100% of CO from this source is transferred to the blood of the consumer (an estimate representing a hypothetical worst-case scenario). According to the authors, such treatment of meat will thus cause only a very minor effect in comparison to what is considered the safety limit, even when assuming maximum uptake of CO. Watts et al. (1978) exposed beef to a gas containing 1% CO for 3 days, and found that this resulted in a CO saturation of approximately 30% of the myoglobin. CO was lost under such storage conditions, with a half-life of approximately 3 days. After cooking, the CO concentration in the meat decreased to

below 0.09 ppm (equivalent to approximately 0.1 mg/kg). Maximum loss after cooking (on burner at 195°C) amounted to approximately 85%.

Comparison of CO Exposure through Air and Meat (CO Treated)

There is little data available for such a comparison, but a rough overview nevertheless provides some points of reference. An adult inhales 10-20m^3 air per 24 hours (depending on the activity level). This is the equivalent of 0.42-0.84m^3 per hour (or 3.36-6.72 m^3 per 8 hours).

To stay within a maximum blood COHb level of 1.5%, the CO concentration in the air must be 24 mg/m^3 for 1 hour at moderate physical activity, at 9.2 mg/m^3 for 8 hours (according to Table 3). In comparison, the CO exposure is 0.1 mg/kg after consumption of 250 g of heated CO-treated meat that has been treated for 72 hours in a gas containing 1% CO (Watts et al., 1978). Table 4 shows a calculation of CO intake from the air and a meal of CO-treated meat.

Table 4: Comparison of CO intake from air within a range without any health impact and theoretical intake of CO through consumption of a meal of CO-treated meat:

Path of Exposure:	CO Intake, 1 hour:	CO Intake, 8 hours
Lungs (15 m^3/24 hours)	24mg x 0.625 = 15.1mg	9.2mg x 5 = 46.0mg
Meat	0.025mg	0.025 mg

For CO balance between air and blood is only achieved after a considerable period of time (hours). The absorption of gases from the intestinal canal to the blood is probably considerably less efficient than from the lungs, where the tissue allows for maximum gas exchange between the alveoli and the bloodstream. This implies that intake of CO through meat probably won't cause any demonstrable increase in the blood CO level (in the form of COHb). And at any rate, the exposure from meat is much lower (approximately one thousand times lower) than through the airways, as shown in the calculations above.

According to the Norwegian Institute of Air Research (SFT 1992), the CO concentration in larger Norwegian cities is on average between 1 and 2 mg/m^3 during the winter. Maximum hourly values have been measured to approximately 60 mg/m^3, and maximum values for 8 hours to about 40 mg/m^3.

Evaluation of Other Gases Used in Foods in the EU

EU's Research Committee on Foods (SCF) has not considered CO. However, the expert team has considered other gases (EUR 1981), such as carbon dioxide (CO_2) and nitrogen oxide (NO). In this connection, the committee employed the following evaluation method, which should be applicable for CO, as well:

CO_2: This compound is a natural product of metabolism, and people are constantly exposed to carbon dioxide from the atmosphere, food and drink. Compared to this exposure, the residual content from its use as an extraction agent is insignificant. Establishing an ADI for this compound is unnecessary. The committee considers this compound acceptable as an extraction agent. It is unnecessary to determine concentration values for the residue.

N_2O: The pharmacological and pharmacokinetic properties of this gas are well known from the extensive use of N_2O as an anaesthetic. Even though no data on residual content are available, such amounts are probably so minor that they are not hazardous to the consumer. The committee finds that it is unnecessary to establish an ADI, and considers the use of N_2O as an extraction agent acceptable.

Toxicological Evaluation of the Use of CO as a Packaging Gas for Meat

People are continually exposed to carbon monoxide, both by means of endogenic production and by inhaled air. Toxicologically, it is the amount of CO bound to the blood hemoglobin (the carboxyhemoglobin percentage) that determines any health effects. The very first effects in sensitive individuals occur at COHb concentrations from approximately 2-3%. To prevent possible health effects even in the most sensitive individuals, a team of Norwegian experts has suggested limits for CO in the air that do not result in COHb concentrations above 1.5%, including the endogenic production at 0.5%. The above-mentioned estimates indicate that even if all CO in the prepared meat is transferred to the consumer's blood, the CO concentration – even a temporary concentration – will remain well below accepted limits in air. From a health perspective, the use of CO in concentrations below 0.5-1 % for fresh meat thus represents no toxicological risk.

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ATTACHMENT 4

Final Report

EVALUATION OF BEEF STEAKS AND GROUND BEEF IN THE PACTIV ACTIVE TECH PACKAGING SYSTEM: EFFECTS OF CARBON MONOXIDE IN THE PACKAGE ATMOSPHERE

for

Pactiv Corporation
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Prepared by:
Kathy Hachmeister, MS
Melvin Hunt, PhD
Weber Hall
Dept of Animal Sciences & Industry
Kansas State University
Manhattan, KS 66506

Phone: 785-532-1232
Fax: 785-532-7059

George Milliken, Ph.D
Dickens Hall
Dept of Statistics
Kansas State University
Manhattan, KS 66506

Phone: 785-532-0514

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PROJECT SUMMARY

The effects of carbon monoxide (CO) in Active Tech modified atmosphere packages (MAP) were determined for:

A. Initial product color,
B. Stability of color during display, and
C. Relationships of color deterioration and microbial populations.

Steaks from three beef cuts (strip loin, tenderloin, and inside round steaks) and ground beef were packaged in a MAP certified gas blend (0.4% CO, 30% carbon dioxide and 69.6% nitrogen) and stored at 35°or 43°F for up to 35 days. Cuts then were removed from MAP and displayed at 34°F until their color was approaching consumer unacceptability. Color and microbial parameters were measured and compared to baseline data of comparable product exposed to oxygen but not CO.

A fundamental goal of this research was to determine if CO extended the color life of beef cuts and ground beef beyond their microbial soundness, i.e., did color mask spoilage.

CONCLUSIONS

- The Active Tech MAP system containing CO in the gas blend produced products that were equally as red as products packaged with traditional oxygen permeable overwrap.
- Improvement in visual appearance especially in the tenderjoin and inner portion of the inside round steaks were observed on day zero of display and throughout display.
- Color of products exposed to CO was a typical, bright red when the outer MAP bag was removed and products were allowed to bloom for 60 to 90 minutes.
- Color declines for products stored in MAP with CO compared well to baseline products exposed to oxygen. Hence, a typical discoloration pattern was seen in both baseline and MAP studies.
- Color life for tenderloin and inside round steaks (and to a lesser extent ground beef) was slightly longer than their baseline counter parts, especially when stored 35°F vs. 43°F.
- Although microbial growth curves changed in slope and exponential growth based on the environment in the packages, bacterial growth was neither encouraged nor suppressed by the addition of CO to the MAP gas blend.
- Aerobic bacteria and facultative anaerobes followed typical patterns of growth contingent upon the environmental conditions.
- Effects of storage temperature (35° vs. 43°F) and increased storage time (21 or 35 days) resulted in typical redness decline, increase in off-odors and microbiological changes.
- CO neither masked spoilage nor resulted in color life extension beyond the point of microbial soundness.

INTRODUCTION

Marketing of case-ready meats has moved beyond the concept stage to reality. This method of delivering meat to retailers is expected to be the predominate system within five years. Some of the largest retailers are already paving the way for this makes-sense marketing system.

Modified atmosphere packaging (MAP) systems are a necessity for case-ready meats because current retail meat over wrapping does not fulfill requirements for shelf life and other needs. Processors can choose either high-oxygen or low-oxygen MAP for retail-ready meats. Both systems rely upon the meat having certain functional properties needed to optimize delivery of cuts with excellent display color life and sound microbial quality.

In low-oxygen MAP, such as the Active Tech System of Pactiv Corporation, it is essential that the meat achieve a stable red color that extends throughout storage and display. This usually is accomplished by modifying the package atmosphere so that the meat pigment returns to its purple-red state (deoxymyoglobin). Then, at display, packaged cuts are re-exposed to oxygen (air) to re-form a bright-red color (oxymyoglobin). Some muscles can easily accomplish this function whereas other muscles have a difficult time – due principally to short comings of their inherent muscle chemistry. Thus, novel ways to aid in obtaining desirable color during storage and display would be beneficial.

Gas atmosphere composition plays a critical role in the functionality and efficacy of MAP systems for meat. The atmosphere affects one or more of the following: product appearance, shelf life, microbial and palatability issues, gas dynamics, purge, and myoglobin functionality. Typical atmospheres for low-oxygen MAP utilize carbon dioxide (CO_2) and/or nitrogen (N_2) prior to the meat being re-exposed to oxygen. Addition of small amounts of carbon monoxide (CO) to a CO_2 and/or N_2 atmosphere could aid in producing a more functional pigment color in MAP, especially in meat cuts known to have lower color stability. CO is well known for its ability to bind to myoglobin and form a bright, crimson-red colored pigment known as carboxymyoglobin. However, carboxymyoglobin is believed to stabilize meat color beyond its microbial shelf life. Consequently, consumers may not be able to rely on color as an indicator of quality at time of purchase. Research is needed to address the use of low levels of CO in a MAP system.

HYPOTHESIS AND OBJECTIVES

This research was based on the hypothesis that a small quantity (<0.5%) of CO combined with the typical gases of MAP (CO_2 and N_2) would produce meat color complimentary to the quality needs of a case-ready meat delivery system without compromising consumer quality issues. More specific objectives evaluated the effects of CO in the Active Tech System for:

- **The initial color of intact muscles and ground beef** – this objective addressed color differences between meat in MAP containing CO vs. packaging in O_2.
- **The color deterioration of these products during display** -- these data defined the color display stability of meat in MAP containing CO vs. packaging in O_2.
- **The microbial profile of the meat stored with or without mild temperature abuse** – this portion provided information about microbial growth with CO in MAP relative to the time-honored relationship between color deterioration and spoilage.

EXPERIMENTAL PROCEDURES

This project involved two phases. The **Baseline Display Study** characterized the color and microbial traits of selected cuts and ground beef using typical oxygen-permeable packaging under typical retail display conditions. The **MAP Display Study** utilized the Pactiv Active Tech Packaging System in combination with a unique, certified gas blend (0.4% CO, 30% CO_2 and 69.6% N_2) in the package atmosphere during storage conditions (pre-display). The outer MAP bag was removed and the products were displayed in the same manner as the baseline samples. All data from the MAP Portion were compared to the Baseline product.

RAW MATERIALS:

Twelve beef strip loins (NAMP #180 containing the *Longissimus* muscle), 18 tenderloins (NAMP #189A containing the *Psoas major* muscle), 12 inside rounds (NAMP #169A containing the *Semimembranosus* muscle), and 6 batches of ground chuck (80% lean) were obtained from a commercial source (Prairieland Processors, Inc., Kansas City, KS) at four to six days postmortem. Vacuum packaged subprimals and trim that were received at the KSU Meats Laboratory had an internal temperature of 34°F and had never been frozen. Prior to product preparation, subprimals were stored at 34°F. This product was allocated to 6 replications (2 each of the strip loins and inside rounds and 3 tenderloins constituted a replication).

PRODUCT PREPARATION AND PACKAGING:

One inch thick steaks cut from each subprimal and ground beef formed into about one-pound blocks (Beef Steaker, Model 600, Hobart Corp., Troy, OH) were placed on Styrofoam trays (17S for strip loins, 4P for inside rounds, 1 for tenderloins, and 2P for ground beef) containing an absorbent pad (Ultra Zap Soakers, Paper Pak Products, La Verne, CA). Product was overwrapped with polyvinyl chloride (PVC) film (23,000ccO_2/m^2/24hrs; Filmco MW4, LinPac, UK or Omnifilm 4P, Huntsman, Salt Lake City, UT) using a mechanical wrapper (Filmizer Model CSW-3, Hobart Corporation, Troy OH) and was assigned randomly to either a **Baseline Display Study** using only PVC-wrapped packages or a **MAP Display Study** using the Active Tech System of Pactiv Corporation. Trays for MAP were placed individually in barrier bags (4.5ccO_2/m^2/24hrs: NXE 1-300, Alec Enterprises, Burnsville, MN) along with an oxygen absorber (MRM-200, Multisorb Technologies, Buffalo, NY) activated using Pactiv Active Tech Activator No.1. Barrier bags were evacuated, flushed with a certified gas blend containing 0.4% CO, 30% CO_2, and 69.6% N_2, and sealed (Freshvac Model A300, CVP Systems, Inc., Downers Grove, IL).

TREATMENTS:

Baseline Display Study: Twelve packages of ground beef and one steak (≤1/8" fat trim) from each subprimal (12 strip loins, 12 inside rounds, 18 tenderloins, and the 6 batches of ground beef), were evaluated in a baseline study to establish the color and microbial parameters for meat never in MAP and exposed only to atmospheric oxygen. These packages were placed in display about 4 hours post-packaging (see display and measurement details below).

MAP Display Study: To test the effects of CO in MAP, one package of each product from each of 6 replications was selected at random for assignment to all possible combinations of two storage temperatures (35 and 43°F) and three storage times (7,14, and 21 days for ground beef and 7, 21, and 35 days for steaks). The lower temperature represented reasonably good industry practice, and the higher temperature represented a mildly abusive storage conditions. The storage times represented current industry practice.

Prior to display (post-MAP), the O_2 and CO_2 levels in the outer barrier bags were measured using a MOCON head space analyzer (Pac Check™ Model 650, MOCON/Modern Controls, Inc., Minneapolis, MN).

DISPLAY CONDITIONS:

Meat samples were placed in simulated retail display at 34 ± 3°F under 1614 lux (150 ± 5 foot candles; Model 201, General Electric, Cleveland, OH) light intensity (Philips, 34 Watt, Ultralume 30) in open-top display cases (Unit Model DMF8, Tyler Refrigeration Corporation, Niles, MI). Cases were programed to defrost two-times per day at 12 hour intervals. Display case temperatures were monitored during display using temperature loggers (Omega Engineering, Inc., Stamford, CT). Display times varied based on product type, initial microbial loads, and storage conditions. Product was removed from display when the color score was deemed unacceptable by a visual panel (a color score of 3.5). Baseline products were displayed 7, 5, 4, and 3 days for strip steaks, inside rounds, ground beef, and tenderloins, respectively.

VISUAL COLOR EVALUATION:

Ten trained visual panelists evaluated color using a five-point scale where 1 = very bright red, 2 = Bright red, 3 = Slightly dark red or tan, 4 = Moderately dark red or tan, and 5 = Extremely dark red or brown. The cut-off score for consumer acceptable color was ≥3.5. Two portions of the inside round muscle were scored separately. The outer 1/3 portion (OSM) and the deep, inner 1/3 portion (ISM). The middle 1/3 area was not scored. The 10 panel scores were averaged for statistical analysis.

INSTRUMENTAL COLOR AND SPECTRAL DATA:

Samples were instrumentally analyzed for lightness (L*), redness (a*), and yellowness (b*) for Illuminant D-65 (daylight) using a HunterLab MiniScan Spectrophotometer (1.25 inch diameter aperture, Hunter Associates Laboratory, Inc., Reston, VA). Multiple readings (2 to 4 depending on cut size) were taken and averaged for statistical analysis on each cut at each testing period.

ODORS:

At the end of display, each package from the MAP Display Study was evaluated for off odors by two experienced panelists using a 5-point scale were 1 = no, 2 = slight, 3 = small, 4 = moderate, and 5 = extreme off odor. A score of 3.5 was assumed to be unacceptable to consumers.

MICROBIOLOGICAL PROCEDURES:

Microbial populations were estimated at the end of MAP storage (day 0 of display) and at the end of display (day of unacceptable color). For each post-display sample, a portion of the surface area (top surface) that had been exposed to light was excised. After each package was opened aseptically, two cores (ca 2 in^2) were removed (approximately 1/8 inch depth), placed in a sterile stomacher bag, and blended two minutes with 0.1% peptone diluent. Serial dilutions of the homogenate were prepared in 0.1% peptone and appropriate dilutions were plated in duplicate on Aerobic Plate Count Petrifilm™ to determine total aerobic bacterial populations and on E. coli Count Petrifilm™ to estimate generic *E. coli* and total coliform bacterial counts. In addition, appropriate dilutions also were plated in duplicate on MRS agar to determine lactic acid bacterial populations. Aerobic Plate Count Petrifilm™ and E. coli Count Petrifilm™ (3M Microbiology Products, St. Paul, MN) were incubated at 90°F for 48 hours prior to enumeration. LAB populations were counted after 48 hours of 92°F incubation in a CO_2 chamber. Microbial detection limits for intact muscle and ground beef were 1.76 count/cm^2 and 5.0 count/gram, respectively.

pH:

pH was determined on intact muscle and ground beef samples collected on the day of production. Ten grams of sample were added to 100 mL of distilled water and blended for two minutes. A standardized pH meter with an electrode was used to measure pH according to the procedure outlined in the Handbook for Meat Chemists.

FAT AND MOISTURE:

Ground beef samples collected on the day of production were analyzed in duplicate for moisture and fat using AOAC procedures 985.14 and 985.15, respectively.

EXPERIMENTAL DESIGN AND STATISTICS:

The design was a randomized complete block with six replications. A replication consisted of 1 to 3 subprimals (number depended on the size of each cut). Steaks cut from the subprimals and separate batches of ground beef trim were randomly assigned to replication and the treatment combinations. Data were analyzed using analysis of variance and significant differences determined using least significant difference tests at $P<0.05$.

SAMPLING TIMES/PARAMETERS MEASURED:

1. **MAP Gas Composition for oxygen and carbon dioxide levels**
   - Subsample of several ActiveTech packages on production day (2-3 hours post-packaging) to verify gas composition being obtained
   - End of MAP storage at two temperatures
2. **Microbiology:**
   - Initial counts for subprimals and ground beef on the day of production
   - End of MAP storage at two temperatures
   - End of display

3. **Visual Color:**
   - Initial color prior to display lighting
   - End of MAP storage at each of two temperatures and after 60 to 90 min bloom at 34°F (equal to 0 time of display)
   - Daily during display
4. **Instrumental Color:**
   - Initial color = After packaging in PVC on production day for baseline data, minimal exposure to light
   - End of MAP storage at each of two temperatures and after 60 to 90 min bloom at 34°F (equal to 0 time of display)
   - Daily during display
5. **Odor:**
   - At end of display (prior to microbial testing)

RESULTS AND DISCUSSION

The Baseline Study: A random selection of all steaks and ground beef packaged in PVC film were placed in display to serve as a baseline for color and microbiological comparisons. Products were expected to have the lowest microbiological load and ideal color stability using traditional packaging and display conditions for products exposed only to atmospheric oxygen. The inherent muscle chemistry responsible for good color life also was optimal. If the product exposed to CO were to have extended meat color life, then it will be compared to the baseline "control" with the "best" possible color.

Color Reference Points: The discussion below involves both visual and instrumental measures of color. Visual scores were considered the "standard" with instrumental color being discussed relative to its agreement or disagreement with the visual panel, ie, did the objective measurements confirm what the color panel saw. Visual scores of ≥3.5 were considered borderline acceptable. When samples reached this discoloration, they were removed from display. Normally, a* values (higher values indicate more redness) are highly correlated to visual appraisal.

Inside round steaks typically are two-toned in color. The inner portion (ISM) is much less color stable compared to the outer portion (OSM). These portions were scored separately since one portion may have acceptable color while the other has unacceptable color that would be discriminated against by consumers resulting in the whole cut being judged

unacceptable in color. The effects of CO on this bi-colored muscle were needed to confirm that color was not excessively extended in either portion.

FAT AND MOISTURE, pH, AND INITIAL MICROBIAL LOAD:

Average fat and moisture contents of the ground beef were 19.5 and 61.6%, respectively. pH of both intact muscles and the ground beef ranged from 5.3 to 5.7. The initial aerobic plate counts and lactic bacteria counts for all products were relatively low and indicative of microbial quality of the raw materials and good sanitation. Furthermore, coliforms and *E. coli* were below the detection limit throughout the study.

GAS COMPOSITION AT END OF MAP:

At the end of MAP storage, each package atmosphere was analyzed for O_2 and CO_2 (Table 1). Only 6 (each from a different treatment combination) of 288 packages were removed from the experiment due to leakage.

INITIAL PRODUCT COLOR AND APPEARANCE:

The color of ground beef and steaks entering display (after MAP storage at 2 temperatures) was an attractive, typical red color. Although there were several significant differences in visual scores and a* values (Table 2 and Figures 1-10 at day 0) for product in CO vs. baseline cuts, the variation in color was usually within ± 0.5 of a color score. In general, the initial color of product exposed to CO was very similar to the color of steaks from the baseline display (never exposed to CO). When differences occurred, they were more related to either storage temperature or postmortem age of the product.

Panelists did not consider the color of product exposed to CO atypical. Cuts exposed to CO generally appeared more uniformly bright-red and would be expected to have high consumer appeal. These results were expected, as CO is known to preferentially form a ligand with the colored pigment (myoglobin) in meat resulting in an intensely red pigment known as carboxymyoglobin. At higher levels of CO (0.4% vs. 0.6 to 1%) than used in this experiment, meat color has been described as being an unusual crimson, bright-red color compared to the normal red of oxymyoglobin.

A critical next question was whether the carboxymyoglobin formed on the surface was more stable than the oxymyoglobin formed in baseline product. Further, did the carboxy

pigment deteriorate in a predictable way that consumers could continue to use visual color to judge freshness or potential spoilage.

COLOR DETERIORATION PROFILE:

Visual panel scores (Figures 1-5) and instrumental color (a* values, Figures 6-10) clearly showed that product exposed to CO during MAP storage had color deterioration during display. As expected, visual scores increased (color deteriorated) and a* values decreased (loss of redness) as days in display increased.

In several instances, color appeared to improve late in display – as indicated by a decrease in visual scores (see ground beef, strips loins and tenderloins at 43°F). These decreases were not a return of redness. Rather the apparent decrease resulted from removal of discolored packages the preceding period, leaving product with less overall discoloration remaining in the case.

In general, the color deterioration profiles followed an expected pattern. Namely, the freshest product (baseline packages) had the most stable, red color and the most days in display needed to reach borderline discoloration (Table 3 scores to 3.5) of all treatments. Exceptions occurred for the inside portion of the inside round and tenderloin products, where the product exposed to CO had slightly more stable color than the baseline product (Table 3). These two muscle areas are well known by retailers as having short color life. Thus, CO appeared to improve color life when the inherent muscle chemistry desired for color was limited.

For product from MAP, the longer the storage time, the faster the deterioration, especially at the higher storage temperature (Tables 2 and 3). For packages stored at 43°F, which was a mildly abusive temperature, color deterioration would be expected to accelerate. This phenomenon also is illustrated in Figures 1-10.

Changes in a* values (and other instrumental measures of color not shown) followed the same pattern of color deterioration observed by the visual panelists. There was no evidence that color shelf life was unexpectedly lengthened by exposure of meat to CO in MAP. The question remaining is whether the color life of product in CO masked spoilage, ie, were microbial counts higher than expected based on the degree of discoloration?

COLOR DETERIORATION AND MICROBIAL GROWTH:

Baseline Display Study: Initial, pre-display microbiological data suggested that the raw materials were fresh and processed using good hygienic practices. For intact cuts, lactic acid bacteria, generic *E. coli*, and total coliform counts were below the detection limit of 1.76 CFU/in^2. Initial, pre-display APC for intact muscles ranged from 1 to 1.63 $\log_{10}$ CFU/in^2. Post-display counts were higher ($P<0.05$) than pre-display APC which was an increase in bacterial proliferation and typical deterioration. However, all product had sufficient microbes to be susceptible to spoilage.

Baseline products were pulled from display when the visual panel scores reached ≥3.5. However, the APC did not exceed 5 $\log_{10}$ CFU/unit as shown in Figures 11-14 and lactic bacterial did not exceed 6 $\log_{10}$ CFU/unit as shown in Figures 15-18. Furthermore, off-odor scores for product at end of display (Table 3) ranged from no to slight off odor. Thus, color life in this base population did not exceed microbial soundness.

MAP Display Study: Similar trends in microbial growth occurred in post-displayed samples stored in MAP compared to baseline products. Microbial patterns for product deterioration are shown in Table 4 and Figures 11-18. Products stored under MAP at a slightly abusive temperature showed, as expected, a more rapid increase ($P<0.05$) in microbial counts compared to samples stored at 35°F. For post-MAP (pre-display) and post-display samples, APC were higher at 43°F than 35°F (Table 4), and during the later days of storage at the higher temperature, differences were more obvious. Significant changes ($P<0.05$) occurred in all cuts and ground beef with the exception of SM. Counts for the SM muscle were lower than expected and no significant changes occurring until day 35 of MAP storage. This suggests that quality products that have been handled in a sanitary fashion can be stored in the MAP system up to 35 days without comprising microbial quality. The APCs for intact strip loin and tenderloin steaks stored at 35°F were lower ($P<0.05$) on all days of display on days 21 and 35 post-MAP than steaks stored at 43°F (Figures 12 and 14). Although products did not show a difference in APCs 7 days post-MAP, those products stored at the higher temperature (43°F) were more inferior 21 and 35 days post-MAP.

Did Color Mask Spoilage? Central to this research was to evaluate the idea that the color of CO treated meat might mask spoilage. Food scientists generally agree that meat

color is seriously discolored when microbial counts approach log 10^6, and that off odors frequently appear at counts of 10^7 to 10^8. Numerous studies of ground beef, frequently the product with the highest counts, show that consumer-purchased retail product often has counts of 10^5 to 10^8.

Visual color scoring was considered as the "standard" for determining the time to remove products from display. Because the visual panel scores were the deciding factor for length of shelf life, the interdependence between visual color and APC, LAB, and odor were considered quite important.

Figures 19-21 show aerobic and lactic bacterial growth and odor scores at the end of display plotted against their corresponding visual color scores. All data observations were summed over storage temperature, storage time, and product type and plotted in one graph. If color masked spoilage, then there should be multiple points in the upper left quadrant of the plot, the area represented by unacceptable microbial counts and off odors but with acceptable color (i.e., scores <3.5). This did not occur with any frequency in any of the three plots. Thus, it does not appear that exposure of meat to CO during extended (up to 35 days at either 35° or 43°F) caused meat color to hide spoilage.

Table 1 - Carbon Dioxide (CO_2) and Oxygen (O_2) Levels in MAP Packages of ground beef (GB) and steaks from strip loins (LD), inside round (SM), and tenderloin (TL).

Meat Cut	Storage Temperature, °F	Storage Time, days	CO_2, %	O_2, %
GB	35	7	28.4	0
GB	43	7	28.7	0
GB	35	14	27.7	0
GB	43	14	28.3	0
GB	35	21	27.4	0
GB	43	21	28.0	0
LD	35	7	33.3	0
LD	43	7	34.2	0
LD	35	21	32.4	0
LD	43	21	31.8	0
LD	35	35	31.1	0
LD	43	35	28.5	0
SM	35	7	28.9	0
SM	43	7	29.7	0
SM	35	21	27.9	0
SM	43	21	27.3	0
SM	35	35	26.8	0
SM	43	35	24.6	0
TL	35	7	34.3	0
TL	43	7	34.8	0
TL	35	21	33.6	0
TL	43	21	32.3	0
TL	35	35	32.5	0
TL	43	35	29.2	0

Table 2 - Means for initial visual color and a* values for beef cuts exposed to carbon monoxide during storage at 35°and 43°F in Active Tech MAP vs. baseline cuts exposed only to oxygen.

Trait	Product	Baseline cuts	Time[d] in Active Tech MAP, days at 35° F		
			7	14 / 21	21 / 35
Initial Visual Color	GB	1.3a	1.6b	1.7b	1.8b
	LD	2.2b	2.5b	1.8a	2.2b
	ISM	1.8ab	2.0b	1.7a	2.0b
	OSM	2.6b	2.6b	1.9a	2.5b
	TL	1.9a	2.0a	1.9a	2.1a
Initial a* Values (redness)	GB	23.4a	25.6b	25.9b	25.6b
	LD	25.8a	25.7a	27.1ab	28.1b
	ISM	28.5a	26.9a	30.0a	29.4a
	OSM	27.4a	27.7a	29.8a	29.5a
	TL	23.6a	27.5b	30.0c	29.3c
			Time[d] in Active Tech MAP, days at 43° F		
Initial Visual Color	GB	1.3a	1.7b	1.8b	2.5c
	LD	2.2a	2.3a	2.1a	2.0a
	ISM	1.8a	1.8a	1.7a	2.4b
	OSM	2.6b	2.2a	2.2a	2.0a
	TL	1.9a	2.0ab	1.8a	2.2b
Initial a* Values (redness)	GB	23.4a	25.7b	25.1b	25.5b
	LD	25.8a	25.5a	28.7b	27.5b
	ISM	28.5a	28.7a	28.6a	27.5a
	OSM	27.4a	27.7a	30.2b	29.4ab
	TL	23.6a	27.8b	28.7b	26.4b

[a-c] Means in the same row with a different letter differ (P<0.05).

[d] Ground beef stored 7, 14, and 21 days, other muscles 7, 21, and 35 days.

Table 3 - Means for days to visual unacceptable visual color (score of 3.5) and odor at end of display for beef cuts exposed to carbon monoxide during storage at 35°and 43°F in Active Tech MAP vs. baseline cuts exposed only to oxygen.

Trait	Product	Baseline cuts	Time[e] in Active Tech MAP, days at 35° F		
			7	14 / 21	21 / 35
Days in display to unacceptable color	GB	3.6c	3.0b	3.0b	2.3a
	LD	6.2c	5.0b	5.2b	3.8a
	ISM	3.2a	4.8c	4.0bc	3.5ab
	OSM	4.8c	3.5b	3.4b	2.6a
	TL	2.6a	3.0b	3.2b	2.8ab
			Time[e] in Active Tech MAP, days at 43° F		
Days in display to unacceptable color	GB	3.6d	3.0cd	2.3b	1.5a
	LD	6.2d	5.0c	3.3b	2.3a
	ISM	3.2b	4.0bc	3.1b	2.0a
	OSM	4.5d	3.0c	2.4b	1.6a
	TL	2.6ab	3.0b	2.3ab	1.7a
			Time[e] in Active Tech MAP, days at 35° F		
Off-odor score[f] at end of display	GB	1.5a	1.9a	2.8b	2.4ab
	LD	1.3a	1.3a	2.3b	2.3b
	SM	1.5a	2.2a	3.0b	3.0b
	TL	1.6a	1.2a	3.1b	3.3b
			Time[e] in Active Tech MAP, days at 43° F		
Off-odor score[f] at end of display	GB	1.5a	3.3a	3.6a	3.9a
	LD	1.3a	2.9a	3.3ab	3.6b
	SM	1.5a	2.2a	3.4b	4.0b
	TL	1.6a	2.7a	3.3b	3.8c

[a-d] Means in the same row with a different letter differ (P<0.05).

[e] Ground beef stored 7, 14, and 21 days, other muscles 7, 21, and 35 days.

[f] Off-odor scale: 1 = none, 2 = slight, 3 = Small, 4 = Moderate, 5 = Extreme.

Table 4 - Means for aerobic plate counts (APC) on beef cuts exposed to carbon monoxide during storage at 35°and 43°F in Active Tech MAP vs. baseline cuts exposed only to oxygen.

Trait	Product	Baseline cuts	Time[e] in Active Tech MAP, days at 35° F		
			7	14 / 21	21 / 35
End of MAP storage APCs, log 10 cfu	GB	2.7a	2.6a	4.7b	5.5b
	LD	.7ab	0.2a	1.4bc	1.7c
	SM	1.0b	0.3a	0.3a	0.3a
	TL	1.3b	0.2a	2.6bc	3.1c
End of display APCs, log 10 cfu	GB	4.3a	4.4ab	5.6b	5.5b
	LD	1.4ab	0.4a	2.9bc	3.4c
	SM	0.6a	0.1a	0.6a	2.0b
	TL	0.3a	1.3b	3.5c	3.4c
			Time[e] in Active Tech MAP, days at 43° F		
End of MAP storage APCs, log 10 cfu	GB	2.7a	4.6b	5.8c	6.0c
	LD	0.7a	1.3ab	3.2c	5.1d
	SM	1.0b	0.1a	0.1a	2.8c
	TL	1.3a	1.6a	3.7b	4.0b
End of display APCs, log 10 cfu	GB	4.3a	5.8b	5.9b	6.1b
	LD	1.4a	1.3a	2.8b	5.3c
	SM	0.6a	0.3a	0.7a	2.5b
	TL	0.3a	3.3b	4.2b	4.6b

[a-d] Means in the same row with a different letter differ (P<0.05).

[e] Ground beef stored 7, 14, and 21 days, other muscles 7, 21, and 35 days.

Figure 6
Ground Beef a* Values (Redness) Deterioration During Display Following Storage

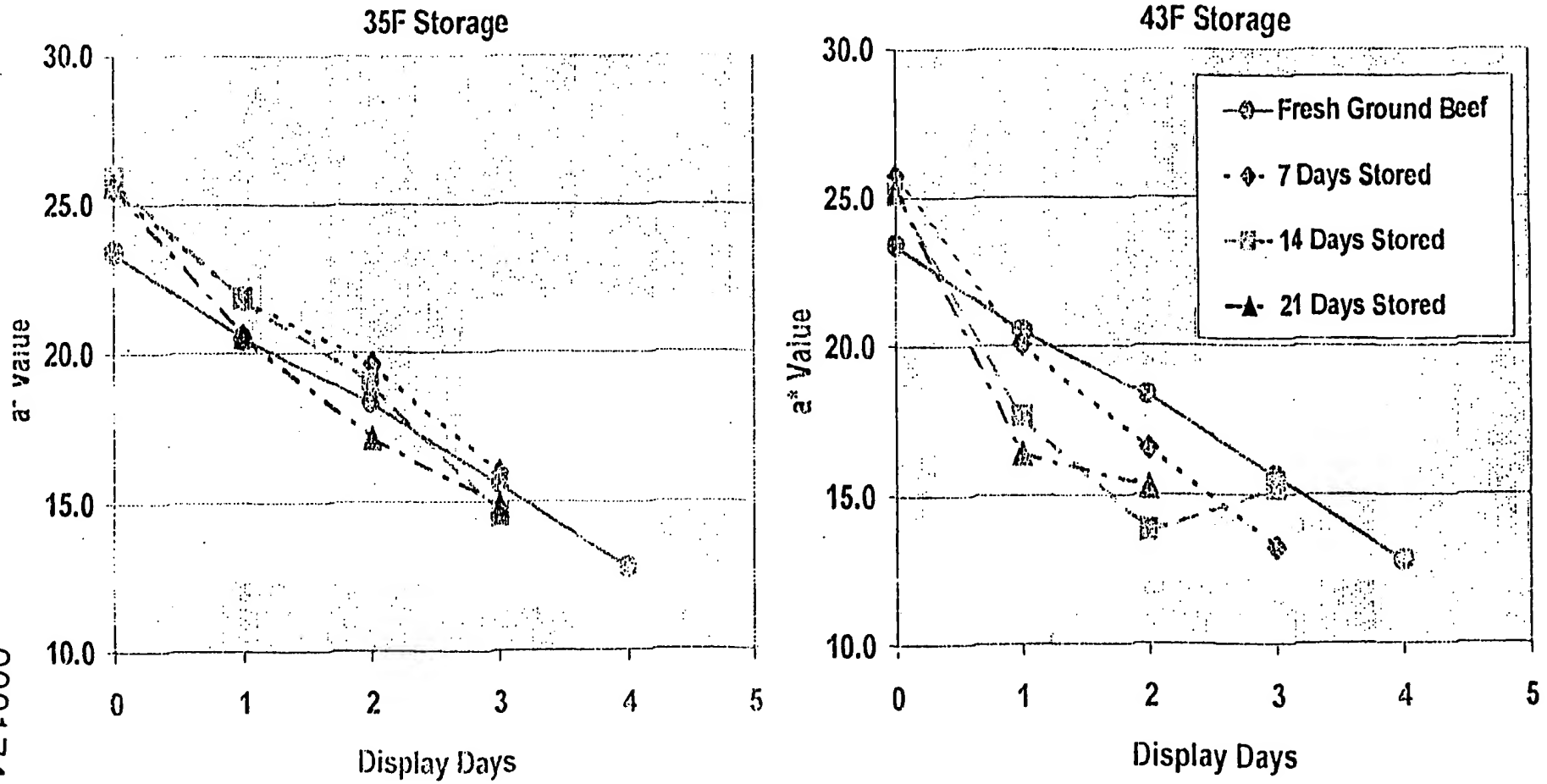


Figure 7
Strip Loin a* Values (Redness) Deterioration During Display Following Storage

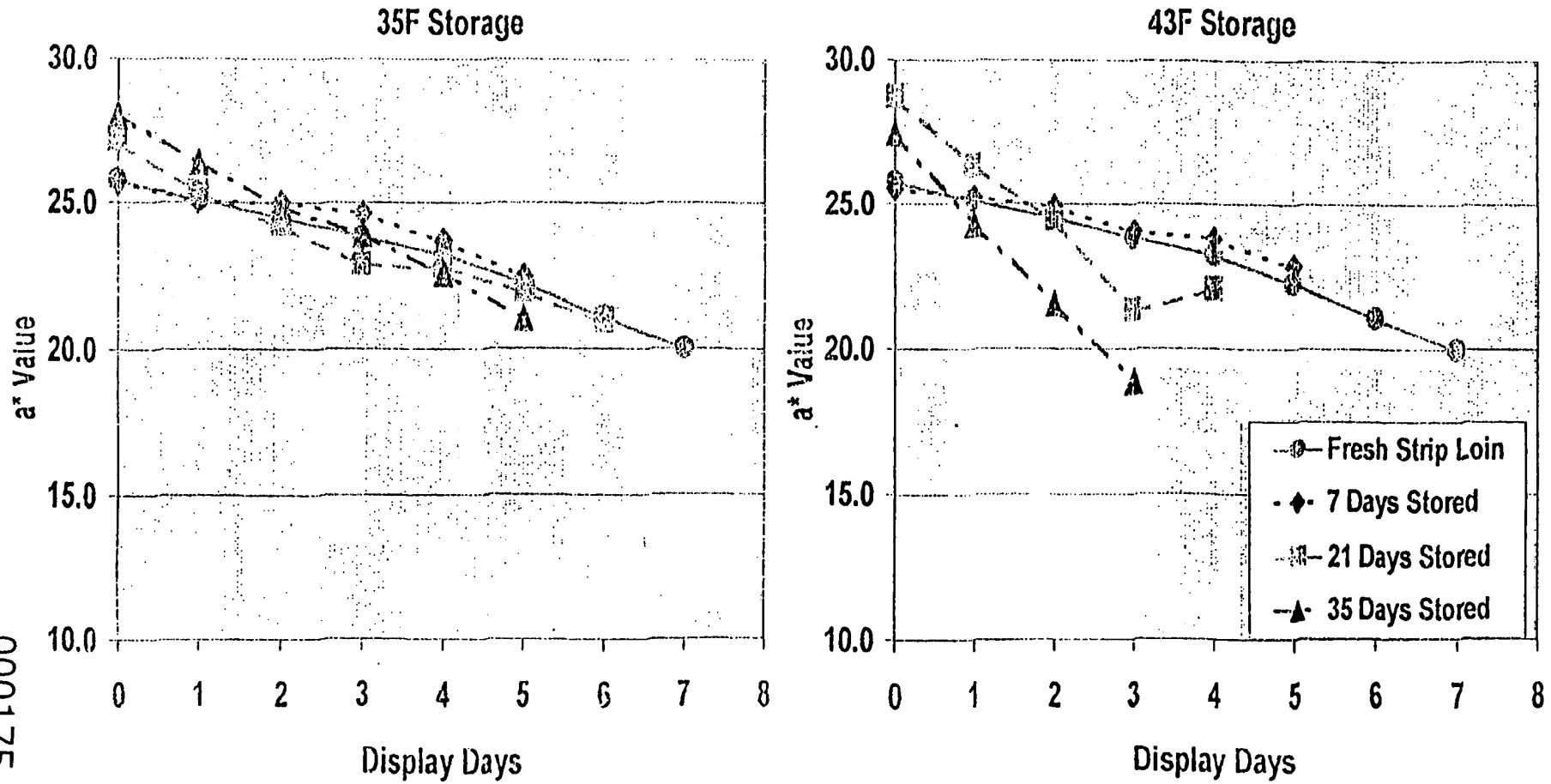


Figure 8
Inside Round (inside portion) a* Values (Redness) Deterioration During Display Following Storage

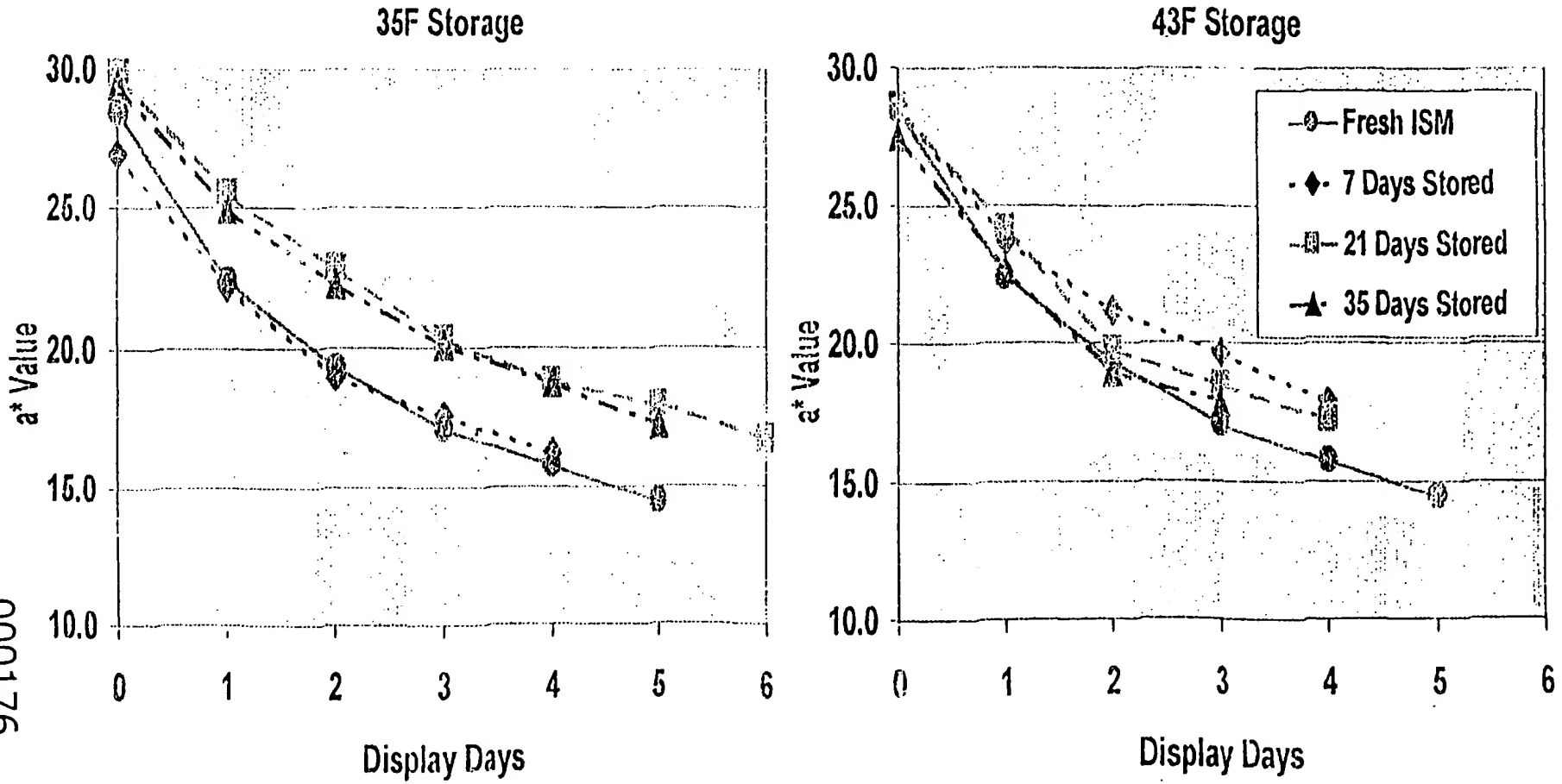


Figure 9
Inside Round (outside portion) a* Values (Redness) Deterioration During Display Following Storage

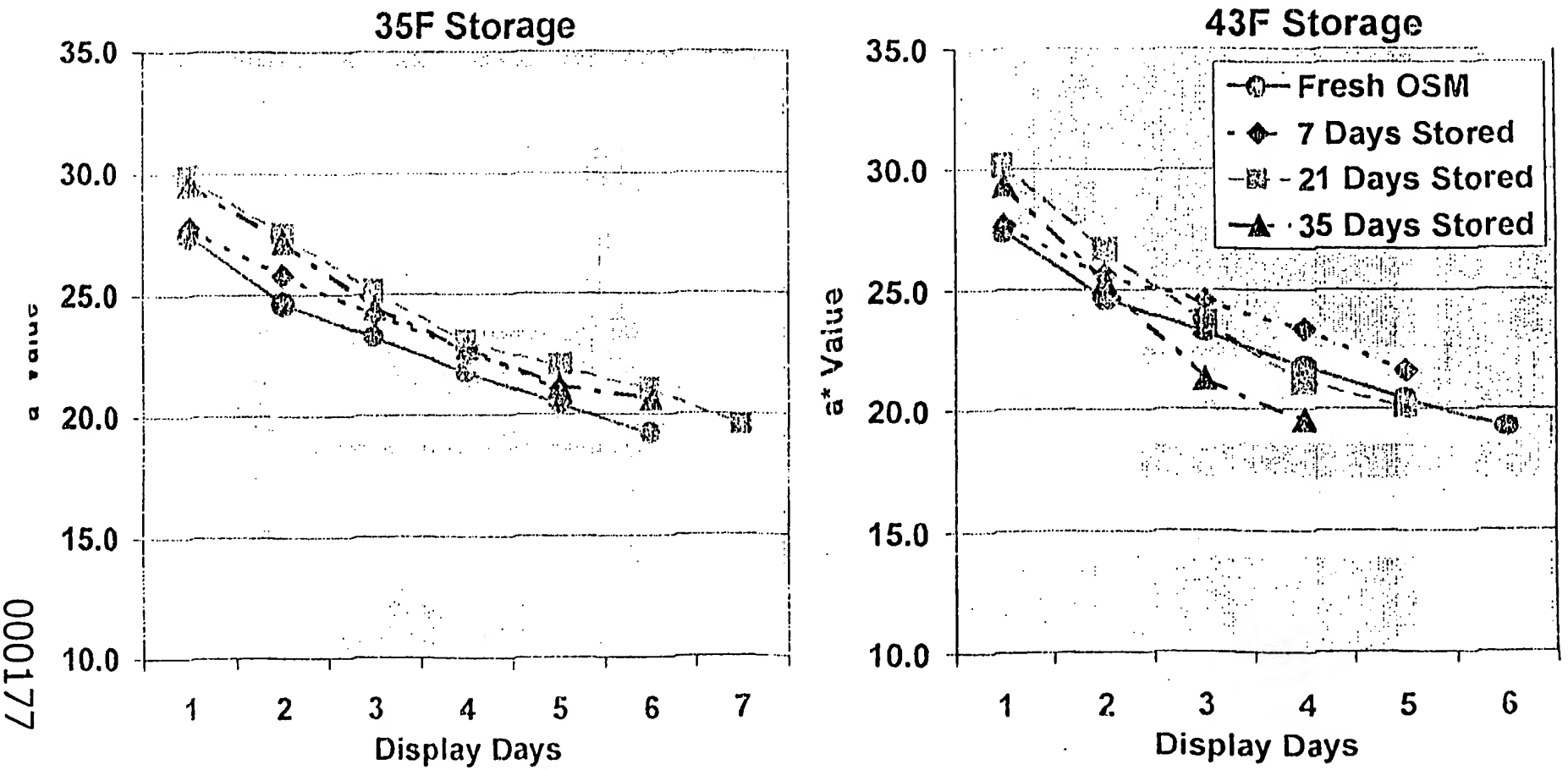


Figure 10
Tenderloin a* Values (Redness) Deterioration During Display Following Storage

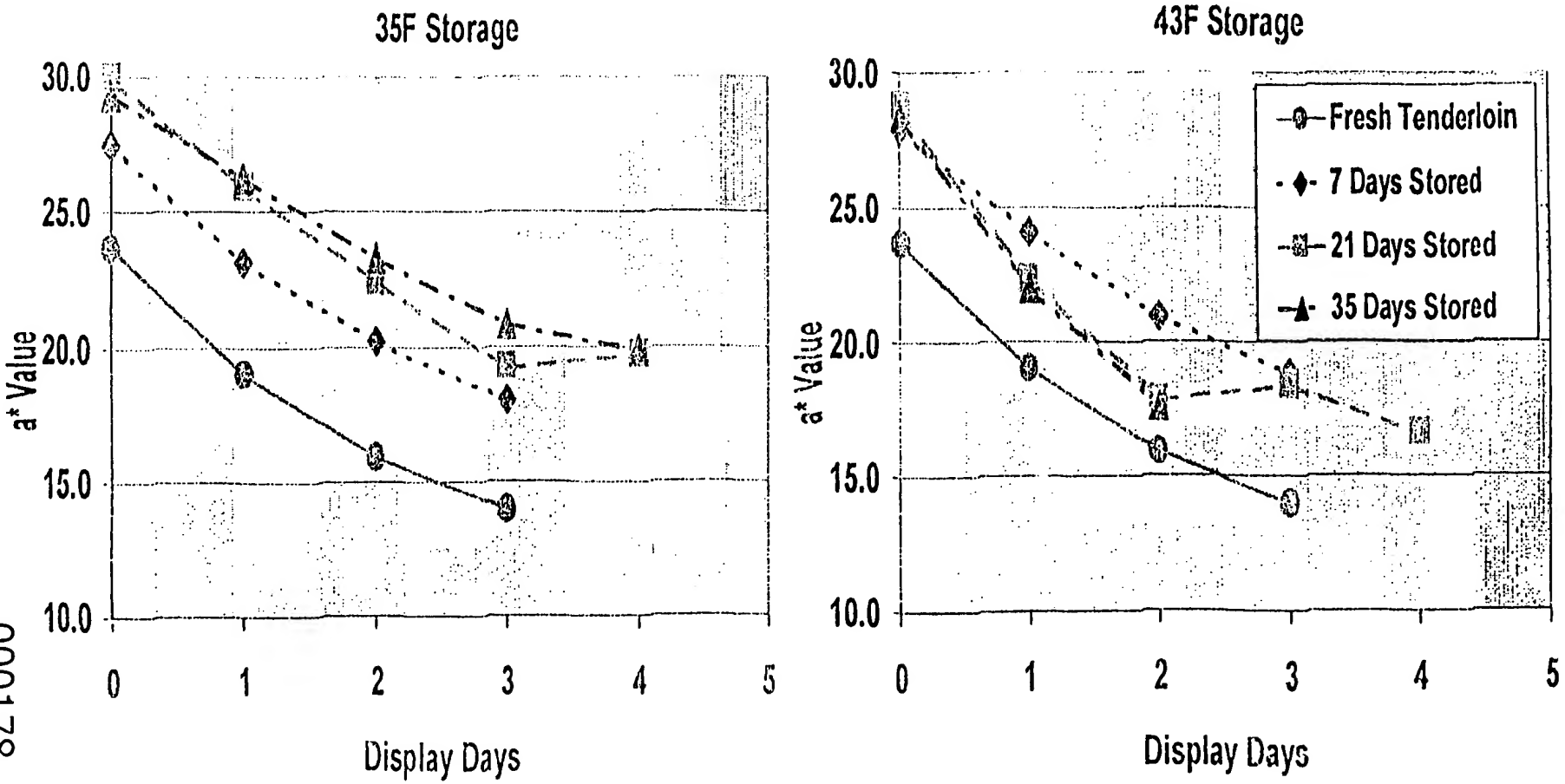


Figure 11
Ground Beef Total Aerobic Plate Counts During Display Following Storage

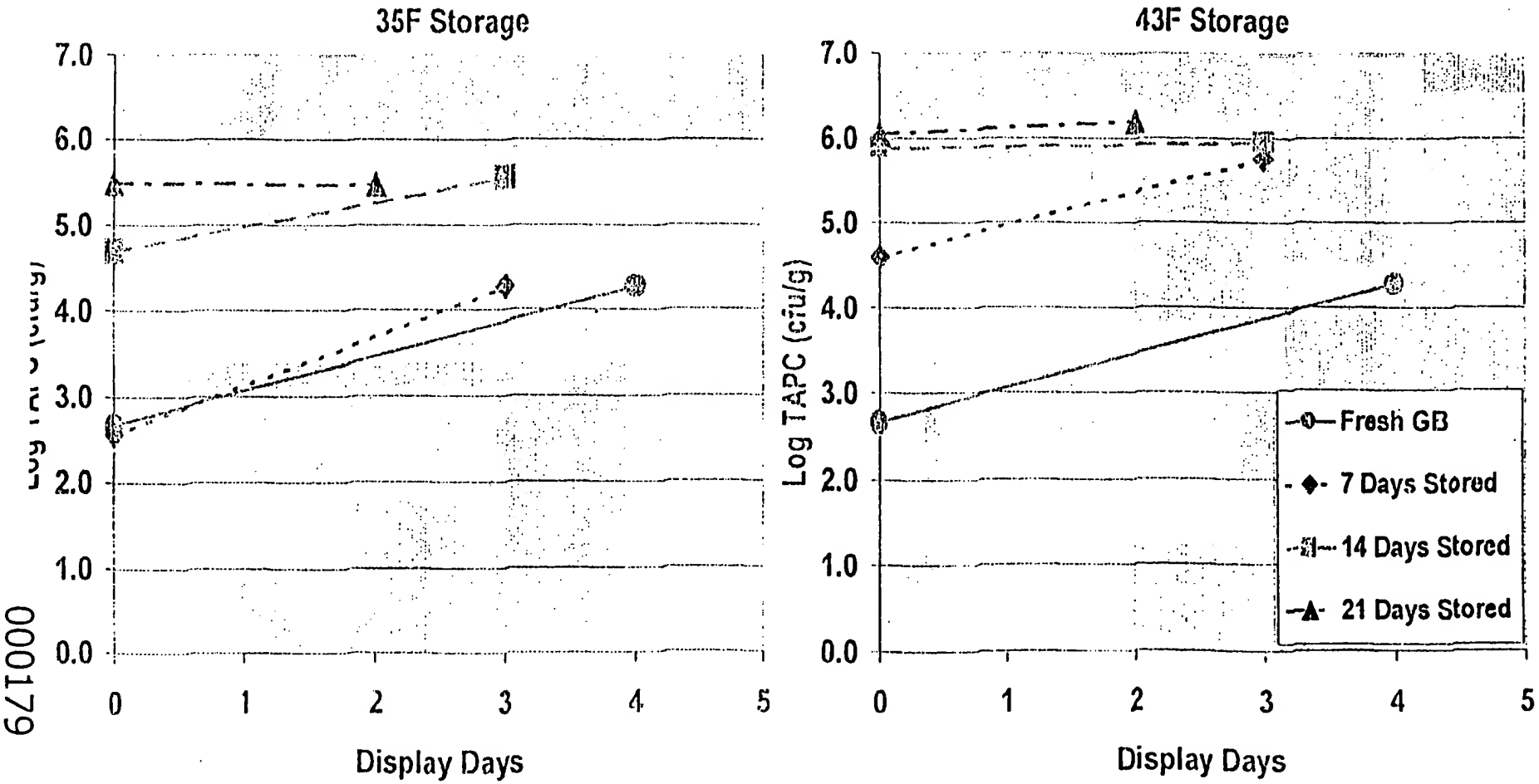


Figure 12
Strip Loin Total Aerobic Plate Counts During Display Following Storage

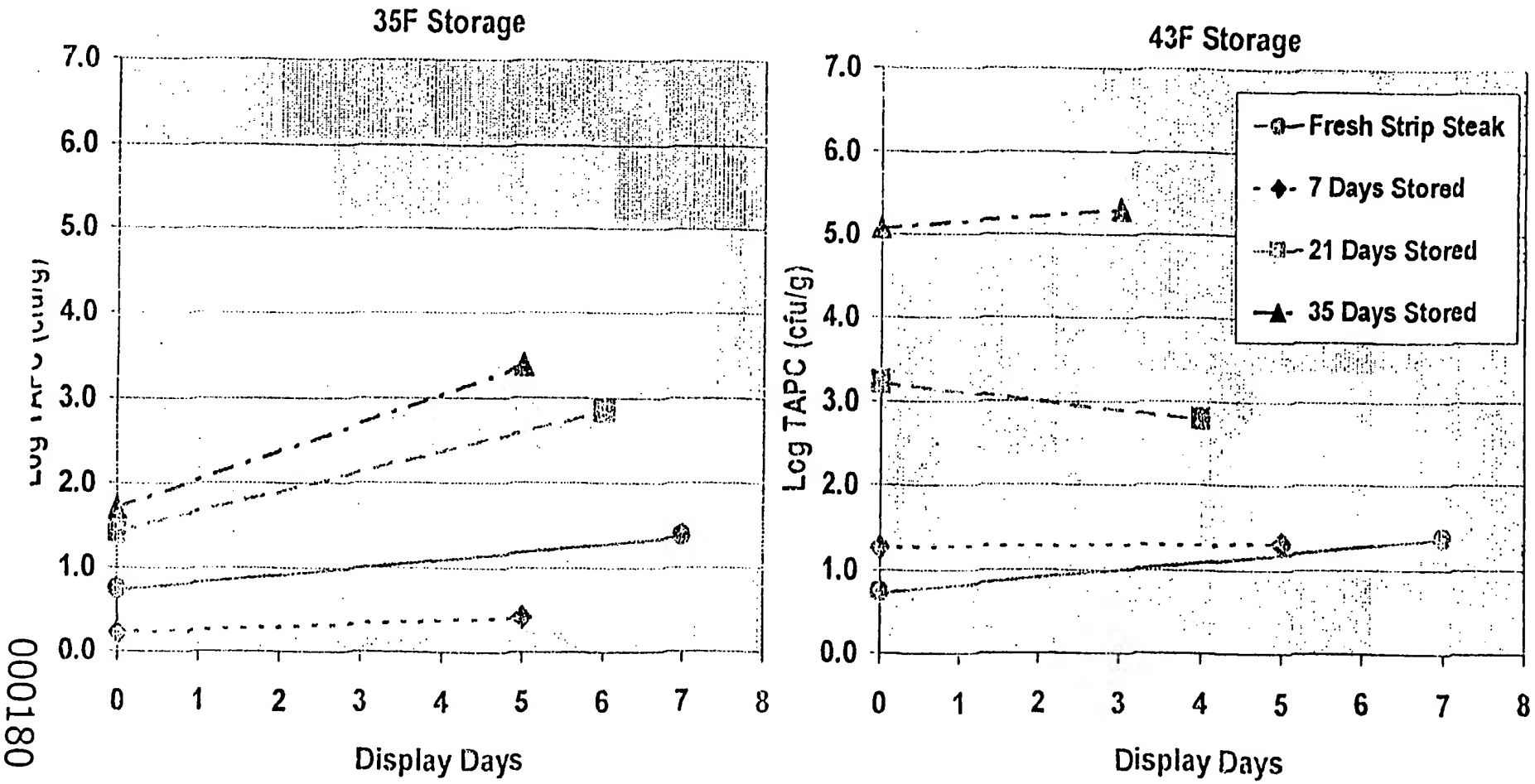


Figure 13
Inside Round Total Aerobic Plate Counts During Display Following Storage

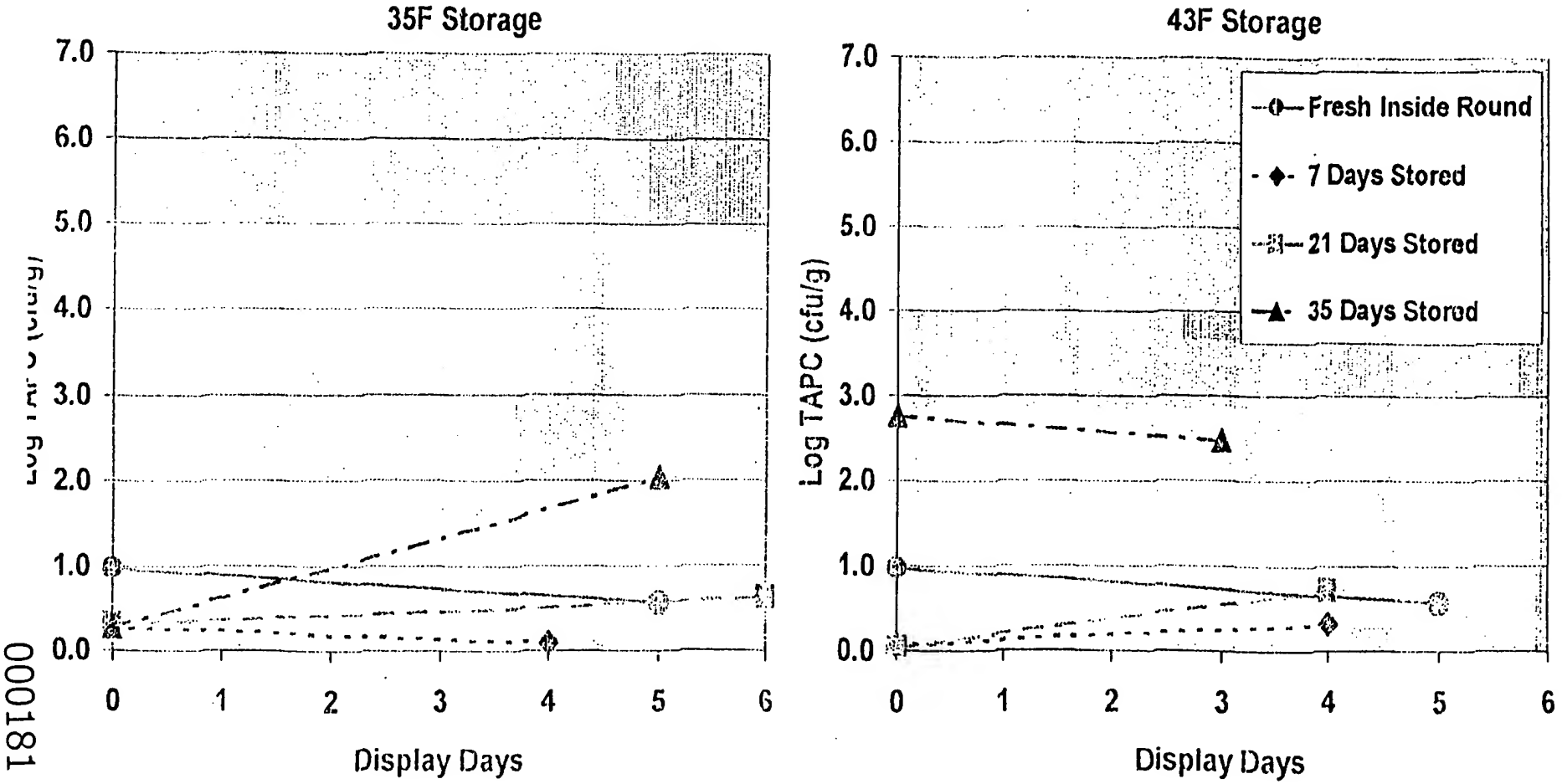


Figure 14
Tenderloin Total Aerobic Plate Counts During Display Following Storage

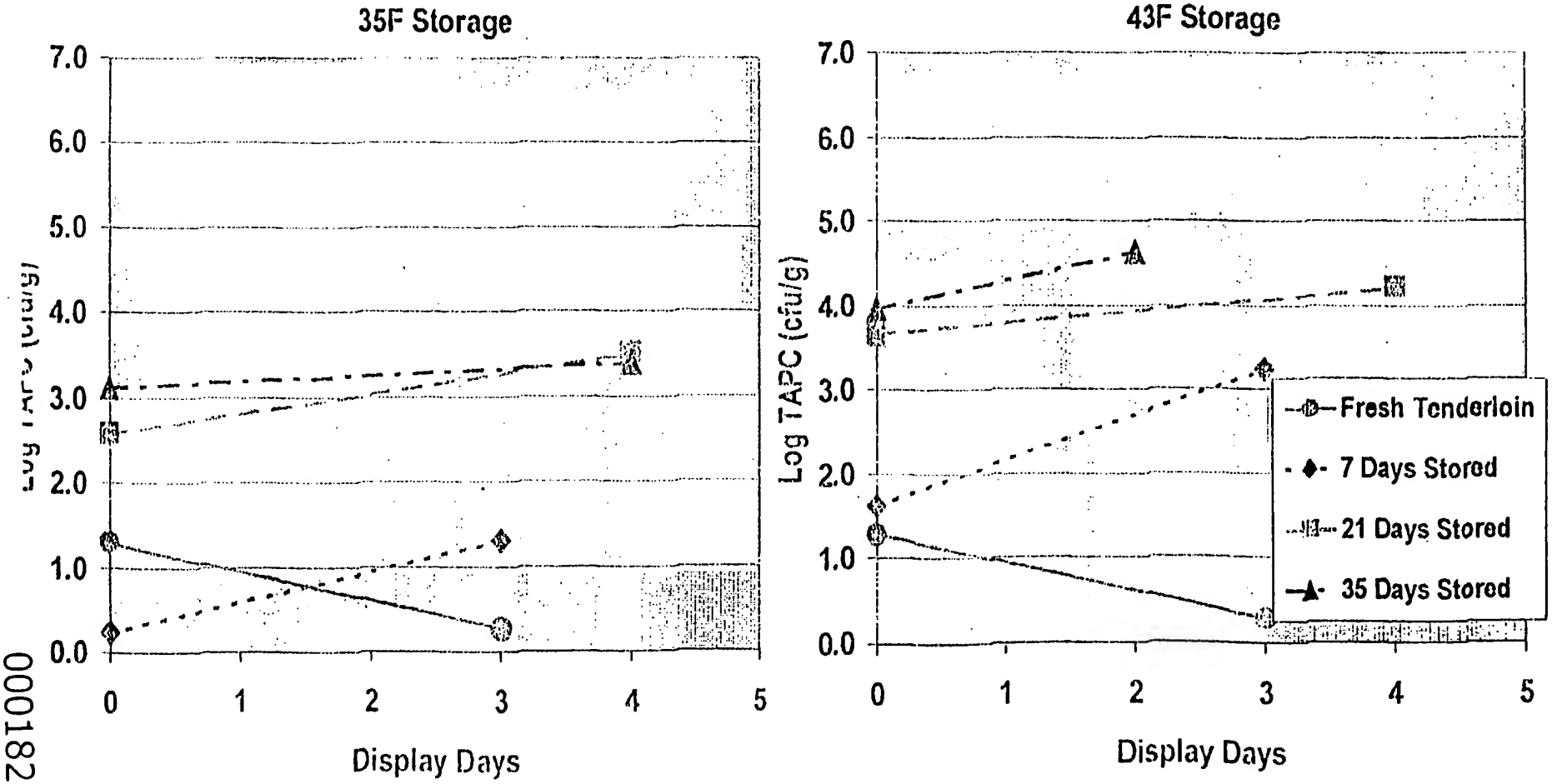


Figure 15
Ground Beef Lactic Acid Bacteria During Display Following Storage

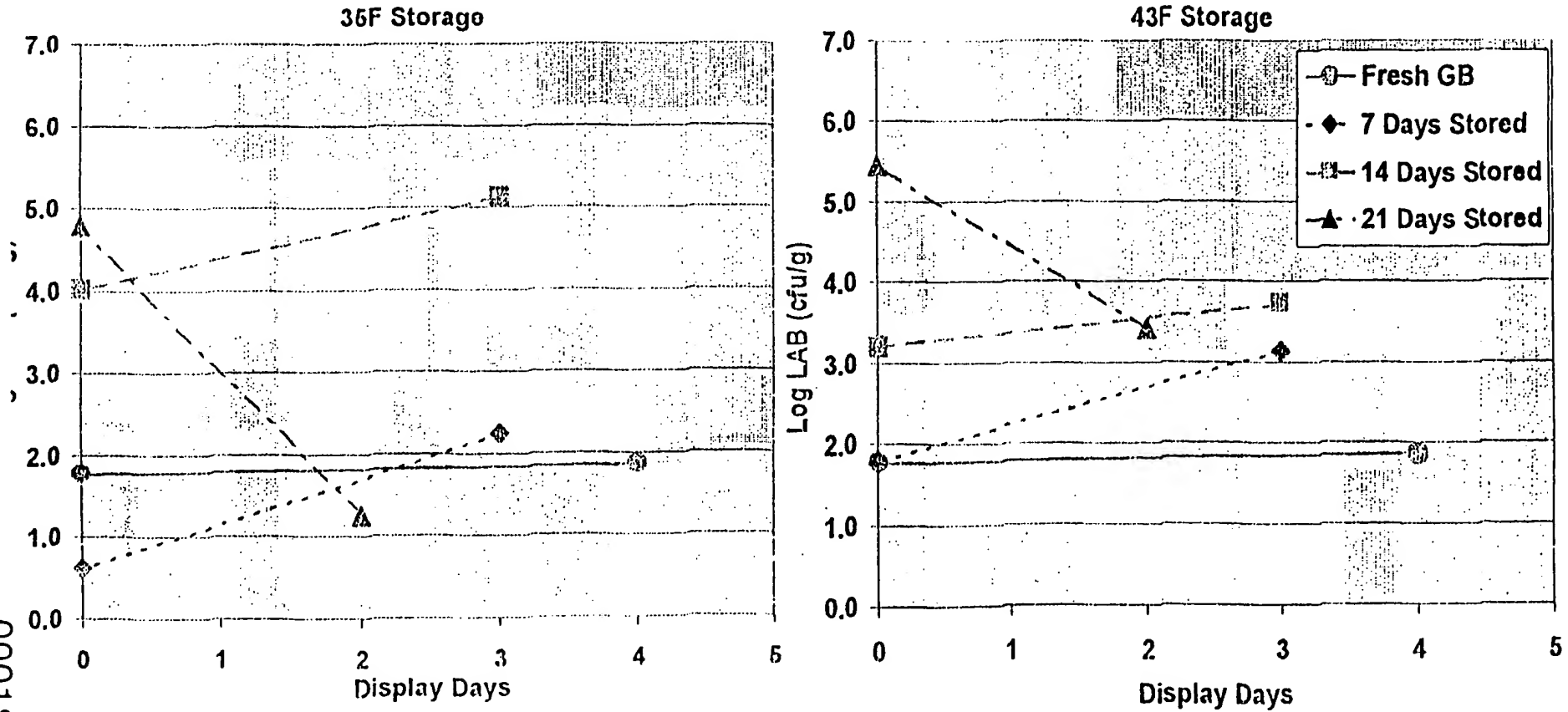


Figure 16
Strip Loin Lactic Acid Bacteria During Display Following Storage

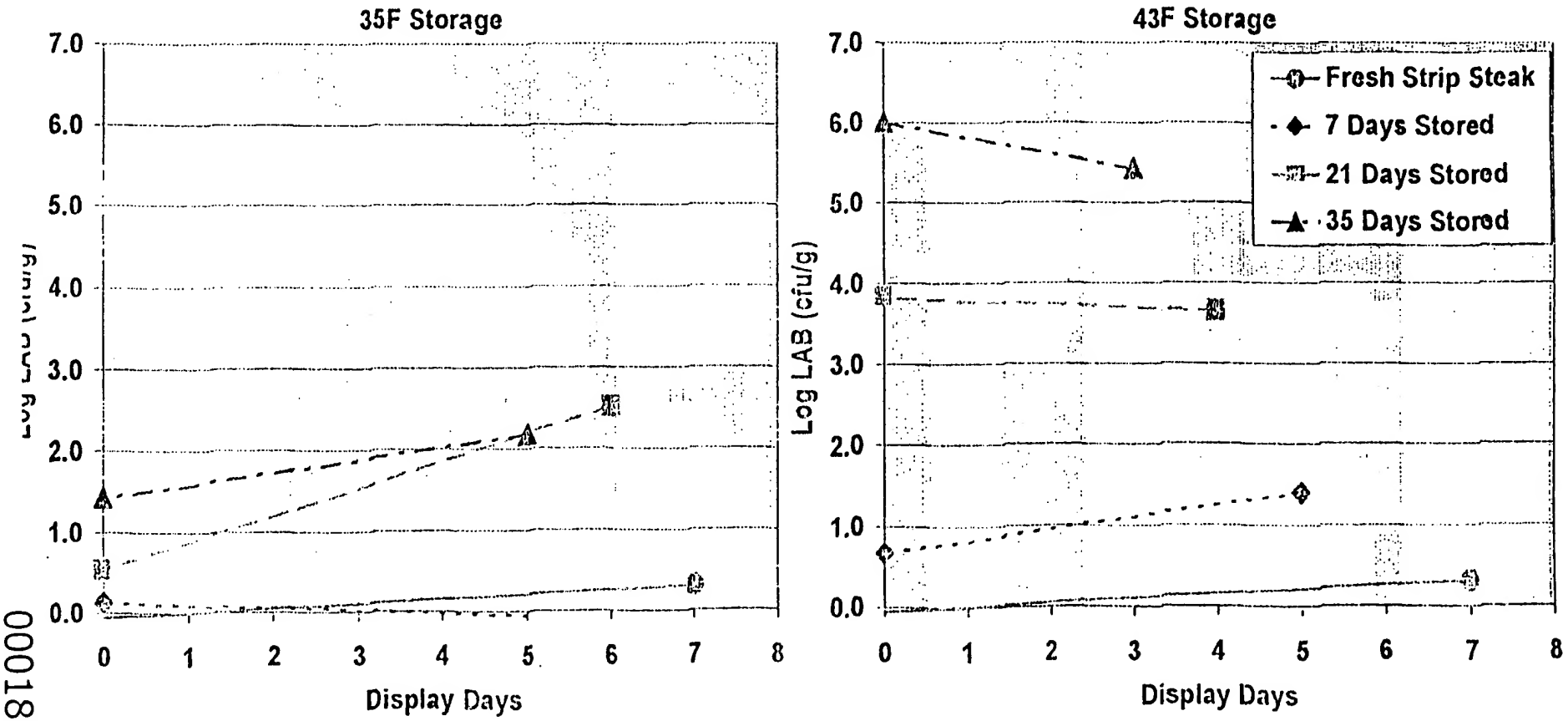


Figure 17
Inside Round Lactic Acid Bacteria During Display Following Storage

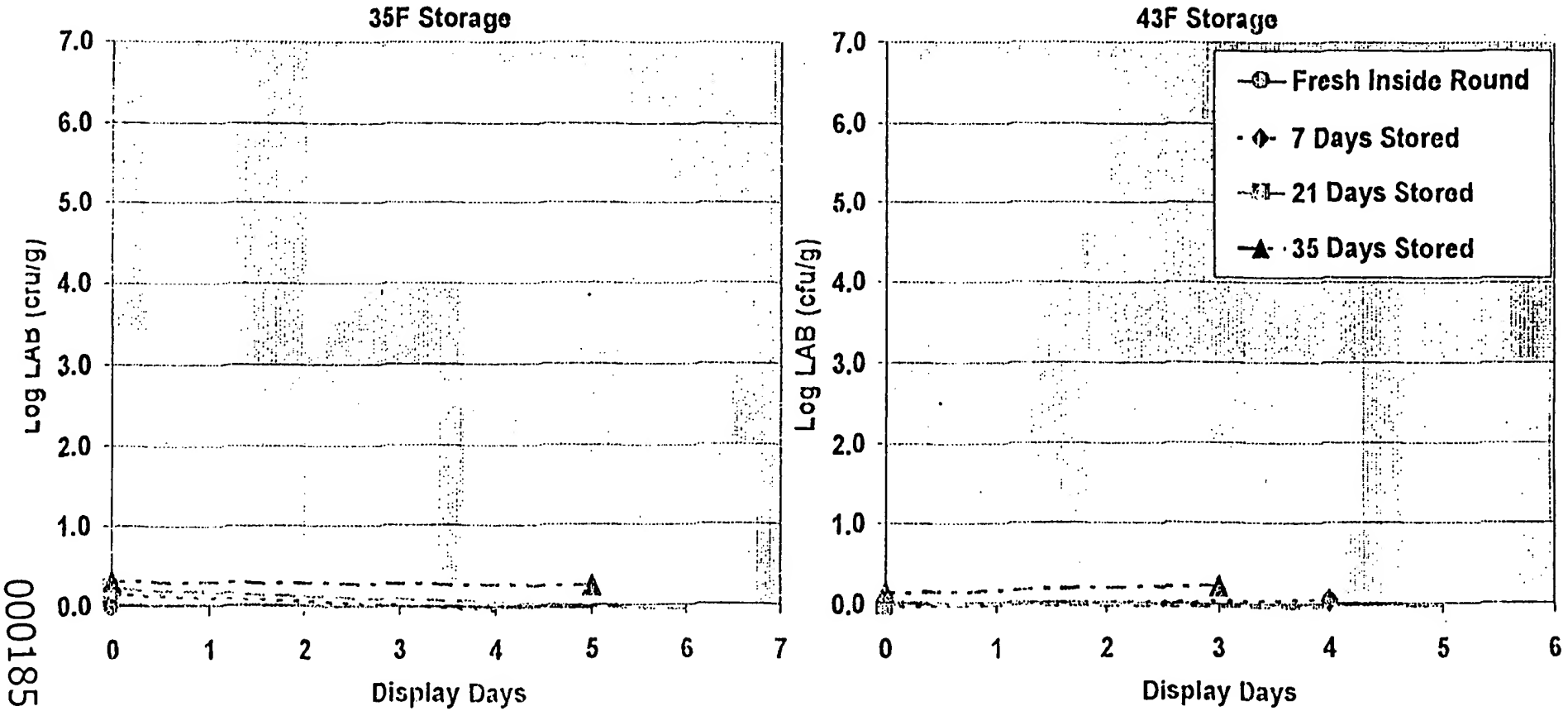


Figure 18
Tenderloin Lactic Acid Bacteria During Display Following Storage

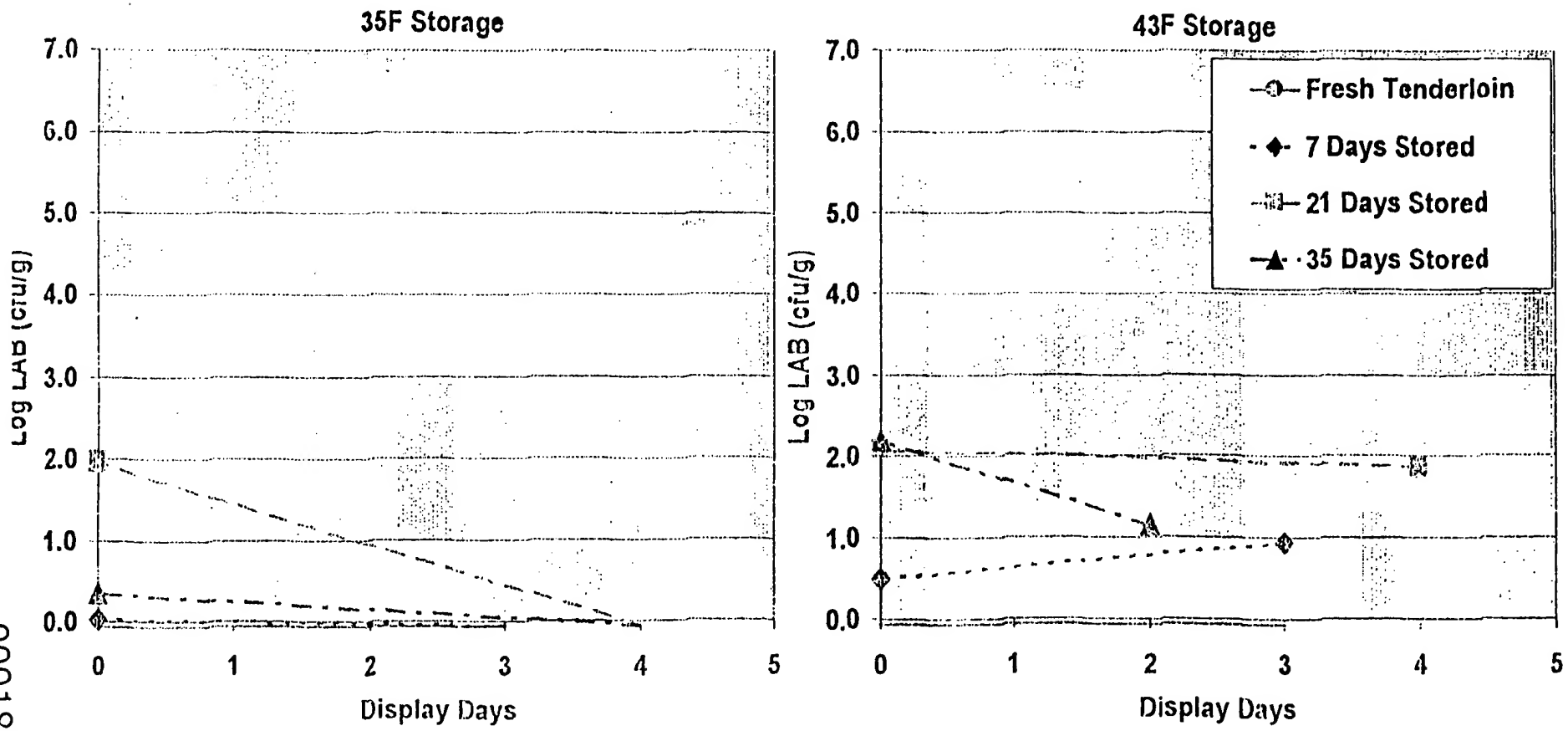


Figure 19
Aerobic Plate Count Log_{10} CFU vs Visual Color

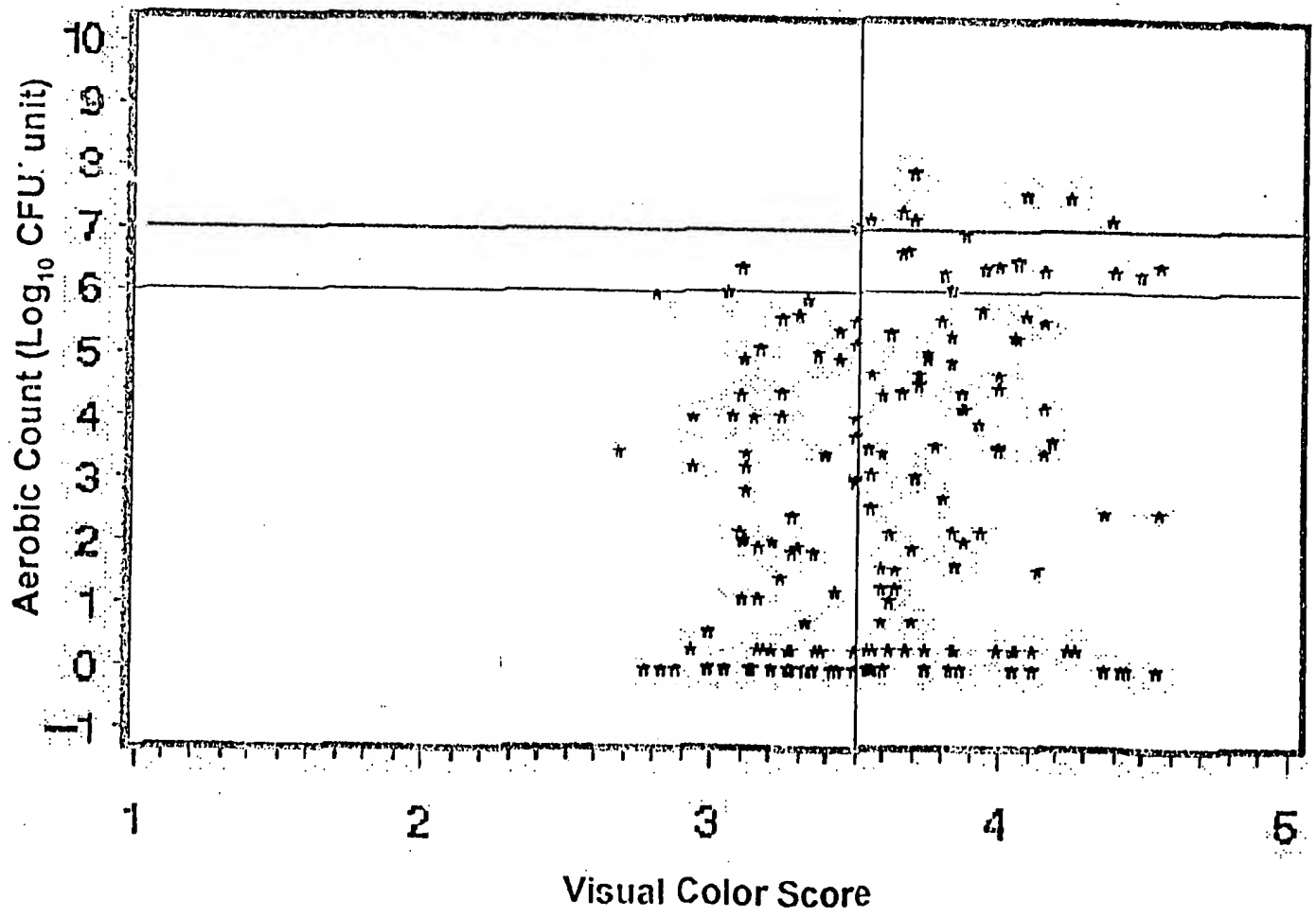


Figure 20
Lactic Acid Bacteria Count Log_{10} CFU vs Visual Color

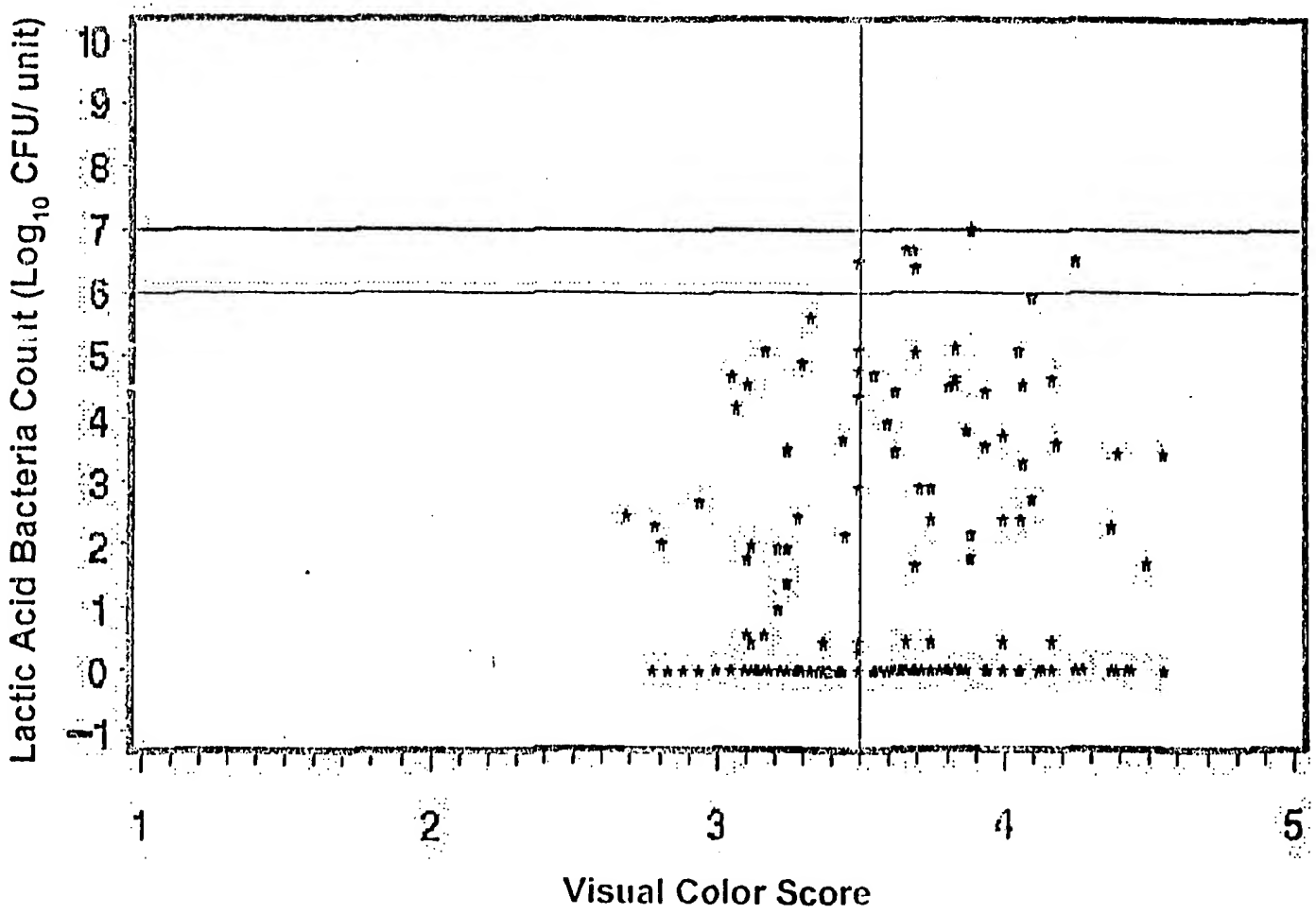
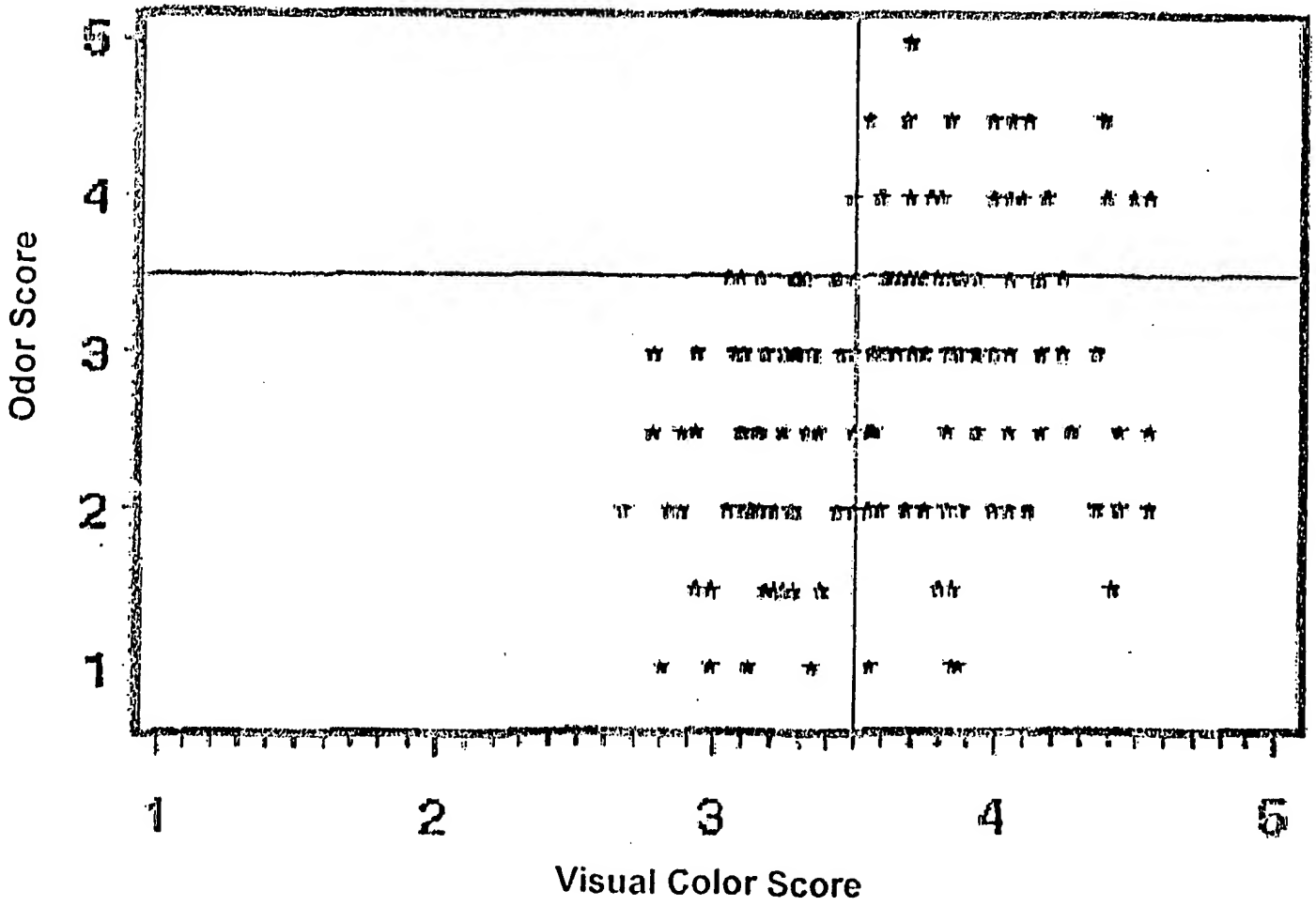


Figure 21
Odor vs. Visual Color



ATTACHMENT 5

HAUN
WELDING SUPPLY, INC.
SPECIALTY GASES DIVISION

Certificate of Conformance

6000 COURT STREET ROAD
SYRACUSE, NEW YORK 13206
(315) 463-5241

6670 MARTIN STREET
ROME, NEW YORK 13440
(315) 339-3378

214 NORTH FOURTH STREET
FULTON, NEW YORK 13069
(315) 592-8012

23791 STATE ROUTE 12
WATERTOWN, NEW YORK 13601
(315) 785-5438

10149 STATE HIGHWAY 56
MASSENA, NEW YORK 13662
(315) 764-6728

311 CLARK STREET
AUBURN, NEW YORK 13021
(315) 252-5398

1 LEE STREET
UTICA, NEW YORK 13502
(315) 724-5382

417 COMMERCE ROAD
VESTAL, NEW YORK 13850
(607) 757-6348

1446 MILITARY TURNPIKE
PLATTSBURGH, NEW YORK 12901
(518) 563-1240

5 GREGORY DRIVE
SOUTH BURLINGTON, VT 05403
(802) 862-4572

48 DORAN AVENUE
GENEVA, NEW YORK 14456
(315) 781-6650

Customer: Pactiv Packaging

Material Submitted: Carbon Monoxide, Research Purity 99.99% min.

Date Reported: 05/08/01

Component	Specification
Carbon Monoxide	99.99% min.
Oxygen	≤ 0.5 PPM
Nitrogen	≤ 10 PPM
Carbon Dioxide	≤ 20 PPM
Methane	≤ 5 PPM
Ethane	≤ 1 PPM
Propane	≤ 1 PPM
Dimethyl Ether	≤ 1 PPM
Hydrogen	≤ 1 PPM
Moisture	≤ 1 PPM

Note: Analysis are conducted utilizing approved analytical method (s) and are correct to within the analytical accuracies of this (these) method (s).

Quality Control Approved Lonnie Nash 05-08-01

CONSISTENLY FULFILLING CUSTOMER'S EXPECTATIONS THROUGH INNOVATIVE SOLUTIONS AND TEAMWORK

ATTACHMENT 6

Designation: D 1946 – 90 (Reapproved 2000)

Standard Practice for Analysis of Reformed Gas by Gas Chromatography[1]

This standard is issued under the fixed designation D 1946; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers the determination of the chemical composition of reformed gases and similar gaseous mixtures containing the following components: hydrogen, oxygen, nitrogen, carbon monoxide, carbon dioxide, methane, ethane, and ethylene.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 260 Practice for Packed Column Gas Chromatography[2]

3. Summary of Practice

3.1 Components in a sample of reformed gas are physically separated by gas chromatography and compared to corresponding components of a reference standard separated under identical operating conditions, using a reference standard mixture of known composition. The composition of the reformed gas is calculated by comparison of either the peak height or area response of each component with the corresponding value of that component in the reference standard.

4. Significance and Use

4.1 The information about the chemical composition can be used to calculate physical properties of the gas, such as heating (calorific) value and relative density. Combustion characteristics, products of combustion, toxicity, and interchangeability with other fuel gases may also be inferred from the chemical composition.

5. Apparatus

5.1 *Detector*—The detector shall be a thermal conductivity type or its equivalent in stability and sensitivity. The thermal conductivity detector must be sufficiently sensitive to produce a signal of at least 0.5 mV for 1 mol % methane in a 0.5-mL sample.

5.2 *Recording Instruments*—Either strip chart recorders or electronic integrators, or both, are used to display the separated components. Although a strip chart recorder is not required when using electronic integration, it is highly desirable for evaluation of instrument performance.

5.2.1 The recorder, when used, shall be a strip chart recorder with a full-range scale of 5 mV or less (1 mV preferred). The width of the chart shall be not less than 150 mm. A maximum pen response time of 2 s (1 s preferred) and a minimum chart speed of 10 mm/min shall be required. Faster speeds up to 100 mm/min are desirable if the chromatogram is to be interpreted using manual methods to obtain areas.

5.2.2 *Electronic or Computing Integrators*—Proof of separation and response equivalent to that for the recorder is required for displays other than by chart recorder.

5.3 *Attenuator*—If manual methods are used to interpret the chromatogram, an attenuator must be used with the detector output signal to keep the peak maxima within the range of the recorder chart. The attenuator must be accurate to within 0.5 % between the attenuator range steps.

5.4 *Sample Inlet System*:

5.4.1 The sample inlet system must be constructed of materials that are inert and nonadsorptive with respect to the components in the sample. The preferred material of construction is stainless steel. Copper and copper-bearing alloys are unacceptable.

5.4.2 Provision must be made to introduce into the carrier gas ahead of the analyzing column a gas-phase sample that has been entrapped in either a fixed volume loop or tubular section. The injected volume must be reproducible such that successive runs of the same sample agree within the limits of repeatability for the concentration range as specified in 11.1.1.

5.4.3 If the instrument is calibrated with pure components, the inlet system shall be equipped to introduce a sample at less than atmospheric pressure. The pressure-sensing device must be accurate to 0.1 kPa (1 mm Hg).

5.5 *Column Temperature Control*:

5.5.1 *Isothermal*—When isothermal operation is used, the analytical columns shall be maintained at a temperature constant to 0.3°C during the course of the sample run and the corresponding reference run.

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[1] This practice is under the jurisdiction of ASTM Committee D-3 on Gaseous Fuels and is the direct responsibility of Subcommittee D03.07 on Analysis of Chemical Composition of Gaseous Fuels.

Current edition approved March 30, 1990. Published May 1990. Originally published as 1946 – 62 T. Last previous edition D 1946 – 82.

[2] *Annual Book of ASTM Standards*, Vol 14.02.

5.5.2 *Temperature Programming*—Temperature programming may be used, as feasible. The oven temperature shall not exceed the recommended temperature limit for the materials in the column.

5.6 *Detector Temperature Control*—The detector temperature shall be maintained at a temperature constant to 0.3°C during the course of the sample run and the corresponding reference run. The detector temperature shall be equal to, or greater than, the maximum column temperature.

5.7 *Carrier Gas*—The instrument shall be equipped with suitable facilities to provide flow of carrier gas through the analyzer and detector at a flow rate that is constant to 1 % throughout the analysis of the sample and the reference standard. The purity of the carrier gas may be improved by flowing the carrier gas through selective filters before its entry into the chromatograph.

5.8 *Columns*:

5.8.1 The columns shall be constructed of materials that are inert and nonadsorptive with respect to the components in the sample. The preferred material of construction is stainless steel. Copper and copper-bearing alloys are unacceptable.

5.8.2 Either an adsorption-type column or a partition-type column, or both, may be used to make the analysis.

NOTE 1—See Practice E 260 for general gas chromatography procedures.

5.8.2.1 *Adsorption Column*—This column must completely separate hydrogen, oxygen, nitrogen, methane, and carbon monoxide. If a recorder is used, the recorder pen must return to the baseline between each successive peak. Equivalent proof of separation is required for displays other than by chart recorder. Fig. 1 is an example chromatogram obtained with an adsorption column.

(1) Because of similarities in thermal conductivities, helium should not be used as the carrier gas for hydrogen when hydrogen is less than 1 % of the sample. Either argon or nitrogen carrier gas is suitable for both percent and parts per million quantities of hydrogen.

(2) The use of a carrier gas mixture of 8.5 % hydrogen and 91.5 % helium will avoid the problem of reversing polarities of hydrogen responses as the concentration of hydrogen in the sample is increased.

(3) The precision of measurement of hydrogen can be increased by using a separate injection for hydrogen, using either argon or nitrogen for the carrier gas.

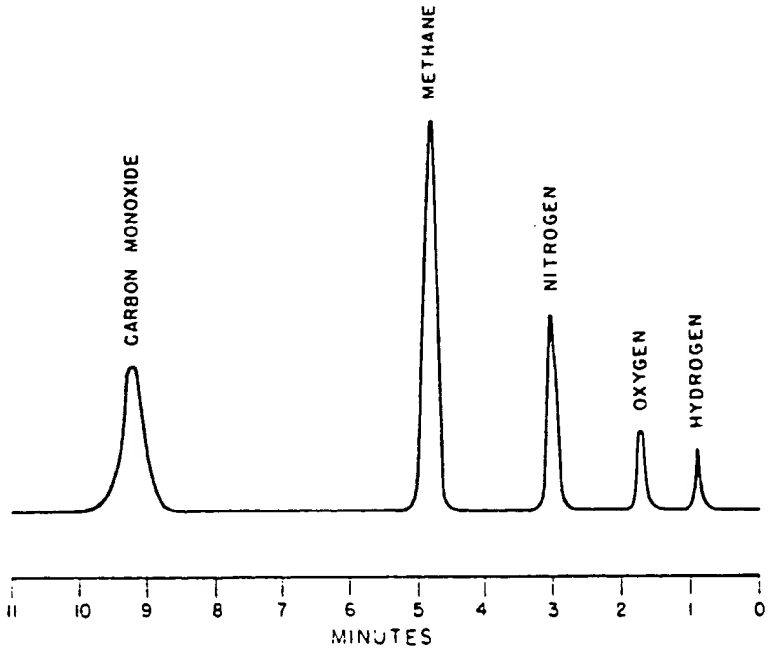
(4) Another technique for isolating the hydrogen in a sample is to use a palladium transfer tube at the end of the adsorption column; this will permit only hydrogen to be transferred to a stream of argon or nitrogen carrier gas for analysis in a second thermal conductivity detector.

5.8.2.2 *Partition Column*—This column must separate ethane, carbon dioxide, and ethylene. If a recorder is used, the recorder pen must return to the baseline between each successive peak. Equivalent proof of separation is required for displays other than by chart recorder. Fig. 2 is an example chromatogram obtained with a partition column.

5.8.3 *General*—Those column materials, operated either isothermally or with temperature programming, or both, may be used if they provide satisfactory separation of components.

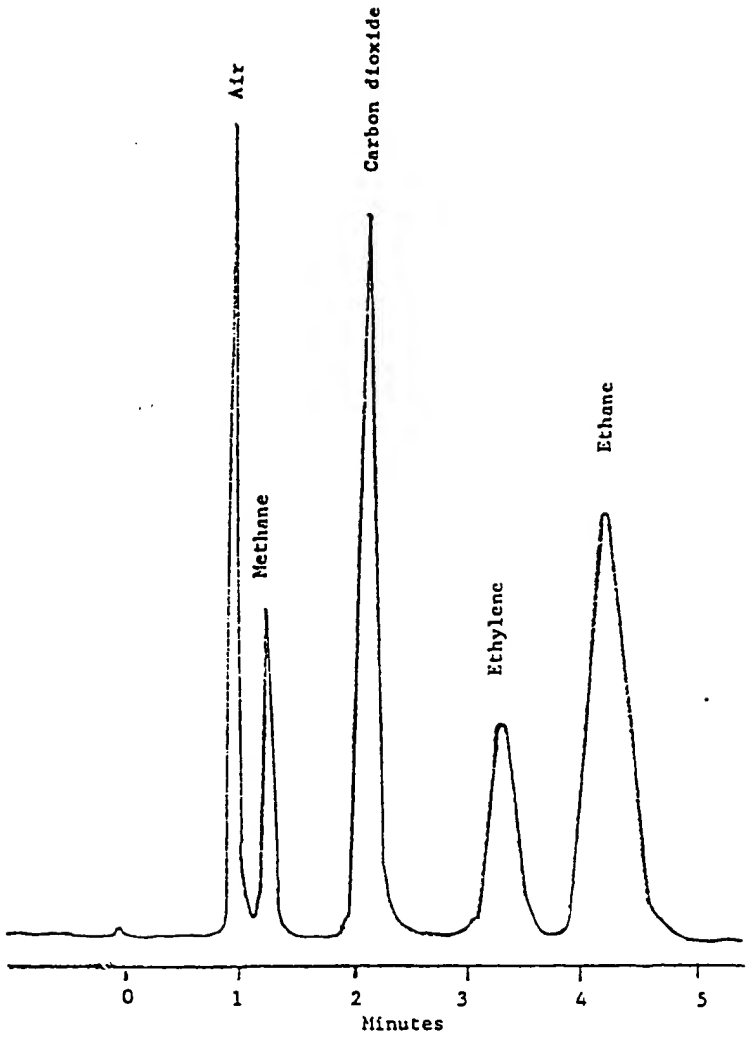
6. Reference Standards

6.1 Moisture-free mixtures of known composition are required for comparison with the test sample. They must contain known percentages of the components, except oxygen (Note



Column: 2-m by 6-mm inside diameter Type 13× molecular sieves, 14 to 30 mesh
Temperature: 35°C
Flow rate: 60-mL helium/min
Sample size: 0.5 mL

FIG. 1 Chromatogram of Reformed Gas on Molecular Sieve Column



Column: 1.2 m by 6.35 mm
Porapak Q, 50 to 80 mesh
Current setting: 225 mA

Temperature: 40°C
Flow rate: 50-mL helium/min
Sample size: 0.5 mL

FIG. 2 Chromatogram of Reformed Gas on Porapak Q Column

2), that are to be determined in the unknown sample. All components in the reference standard must be homogeneous in the vapor state at the time of use. The fraction of a component in the reference standard should not be less than one half of, nor differ by more than 10 mol % from, the fraction of the corresponding component in the unknown. The composition of the reference standard must be known to within 0.01 mol % for any component.

NOTE 2—Unless the reference standard is stored in a container that has been tested and proved for inertness to oxygen, it is preferable to calibrate for oxygen by an alternative method.

6.2 *Preparation*—A reference standard may be prepared by blending pure components. Diluted dry air is a suitable standard for oxygen and nitrogen.

NOTE 3—A mixture containing approximately 1 % of oxygen can be prepared by pressurizing a container of dry air at atmospheric pressure to 20 atm (2.03 MPa) with pure helium. This pressure need not be measured precisely, as the fraction of nitrogen in the mixture such prepared must be determined by comparison to nitrogen in the reference standard. The fraction of nitrogen is multiplied by 0.280 to obtain the fraction of oxygen plus argon. Argon elutes with oxygen in the molecular sieves column. Do not rely on oxygen standards that have been prepared for more than a few days. It is permissible to use a response factor for oxygen that is relative to a stable component.

7. Preparation of Apparatus

7.1 *Column Preparation*—Pack a 2- to 3-m column (6-mm inside diameter stainless steel tubing) with Type 13× molecular sieves, 14 to 30 mesh, that have been dried 12 h or more at 300 to 350°C. Pack a second column (1 m by 6 mm) with Porapak Q,[3] 50 to 80 mesh, that has been dried 12 h or more at about 150°C. Shape the columns to fit the configuration of the oven in the chromatograph.

[3] Available from Waters Associates, Inc., Framingham, MA 01701.

NOTE 4—Variations in column material, dimensions, and mesh sizes of packing are permissible if the columns produce separations equivalent to those shown in Fig. 1 and Fig. 2. Better performance may be obtained by using a 2.1-mm stainless steel tubing with corresponding smaller mesh packing materials and substituting Haysep Q for Porapak Q.

7.2 *Chromatograph*—Place the proper column and sample volume in operation for the desired run in accordance with 8.1 and 8.2. For isothermal operation, the column should be maintained at a temperature between 30 and 45°C. When appropriate, column temperatures may be increased. Adjust the operating conditions and allow the instrument to stabilize. Check the stability by making repeat runs on the reference standard to obtain reproducible peak heights as described in 5.4.2 for corresponding components.

8. Procedure

8.1 *Sample Volume*—The sample introduced into the chromatographic column should have a volume between 0.2 and 0.5 mL. Sufficient accuracy can be obtained for the determination of all but the very minor components with this sample size. When increased sensitivity is required for the determination of components present in low concentrations, a sample size of up to 5 mL is permissible. However, components whose concentrations are in excess of 5 % should not be analyzed by using sample volumes greater than 0.5 mL.

8.2 *Chromatograms*:

8.2.1 *Adsorption Column* (Fig. 1)—Obtain a steady baseline on the recorder with a constant carrier gas flowrate appropriate to the column diameter. Introduce a sample of the unknown mixture at atmospheric pressure into the chromatograph and obtain a response similar to that of Fig. 1 of the components hydrogen, oxygen, nitrogen, methane, and carbon monoxide, which elute in that order. Repeat with a sample of the reference standard. If oxygen is present in the mixture, run a sample of air, either at an accurately measured reduced pressure, or air freshly diluted with helium, so that the partial pressure of oxygen is approximately equal to that of the oxygen in the mixture being analyzed.

NOTE 5—The peak for carbon monoxide can appear between those of nitrogen and methane if the molecular sieves have become contaminated. If this occurs, replace or regenerate the column packing by heating in accordance with 7.1.

8.2.2 *Partition Column* (Fig. 2)—Establish a steady baseline with the helium carrier gas flowing through the Porapak Q column. Introduce a sample of the reference standard and then a sample of the unknown mixture. Obtain responses similar to that shown in Fig. 2 for carbon dioxide, ethane, and ethylene.

8.2.3 All chromatograms for manual measurement should be run at a sensitivity setting that permits maximum peak height to be recorded for each component.

8.2.4 Column isolation valves may be used to make the entire analysis with a single injection if the separations specified in 5.8.2.1 and 5.8.2.2 are produced.

9. Calculation

9.1 The number of significant digits retained for the quantitative value of each component shall be such that accuracy is neither sacrificed nor exaggerated. The expressed numerical value of any component in the sample should not be presumed to be more accurate than the corresponding certified value of that component in the calibration standard.

9.2 *Manual Measurement*—Measure the response of each component, convert to the same sensitivity for corresponding components in the sample and reference standard, and calculate the mole percent of each component in the sample as follows:

$$C = (A/B)(S) \quad (1)$$

where:

C = mole percent of the component in the sample,
A = response of the component in the sample,
B = response of the component in the standard at the same sensitivity as with A, and
S = mole percent of the component in the reference standard.

9.3 If a helium-diluted air mixture was run for oxygen calibration, calculate the fraction of oxygen in the mixture from the fraction of the nitrogen and the composition of the diluted air. Calculate the fraction of nitrogen in the mixture in accordance with 9.1, using the nitrogen response of the reference standard for comparison. Air composition values of 78.1 % nitrogen and 21.9 % oxygen should be used, as argon (0.9 % in air) elutes with oxygen on the molecular sieves column.

9.4 If air has been analyzed at reduced pressure to calibrate for oxygen, correct the equation for pressure as follows:

$$C = (A/B)(S)(P_a/P_b) \quad (2)$$

where:

P_a = absolute pressure at which air was analyzed and
P_b = barometric pressure when sample was analyzed, with both pressures being expressed in the same units.

9.5 Normalize the mole percent values by multiplying each value by 100 and dividing by the sum of the original values. The sum of the original values should not differ from 100.0 % by more than 1.0 %.

10. Analysis of the Reference Standard

10.1 If the composition of the reference standard is not known to a sufficient degree of accuracy, analyze it by the use of pure components for calibration. Obtain chromatograms of the standard as described in 8.2, except measure the pressure of each sample introduced to 0.133 kPa (1 mm Hg). When each chromatogram is obtained, calibrate each component by introducing a sample of the pure component at a pressure that closely approximates its partial pressure in the blend (for example, a component whose concentration in the standard is 50 % is analyzed at 50 % of the pressure at which the standard was analyzed). Use a minimum pressure of 0.665 kPa (5 mm Hg) for minor components. Repeat the analysis with the reference standard. Corresponding peak heights should agree within 1 mm or 1 % (whichever is larger) when recorded on a sensitivity setting that allows maximum response on the recorder chart.

10.2 Calculate the composition of the reference standard by the adjustment of responses of like components to the same sensitivity and calculate the concentration of each component as follows:

$$C = \frac{(100)(R)(P_p)}{(P)(P_r)} \quad (3)$$

where:

C = component concentration, mole percent;
R = response of the component in the reference standard;
P = response of the pure component;
P_p = pressure at which the pure component was analyzed; and
P_r = pressure at which the reference standard was analyzed, with both pressures being expressed in the same absolute units.

10.2.1 Normalize all values as described in 9.4.

11. Precision

11.1 The following data should be used to judge the acceptability of the results:

11.1.1 *Repeatability*—Duplicate results by the same operator should not be considered suspect unless they differ by more than the following amounts:

Component, mol %	Repeatability
0 to 1	0.05
1 to 5	0.1
5 to 25	0.3
Over 25	0.5

11.1.2 *Reproducibility*—Results submitted by different laboratories should not differ by more than the amounts given in 11.1.1 when the same reference standard is used for calibration and the same composition is used for calculations. If calibration is made with pure components or with different reference standards, results submitted by each of two laboratories should not be considered suspect unless the results differ by more than the following amounts:

Component, mol %	Reproducibility
0 to 1	0.1
1 to 5	0.2
5 to 25	0.5
Over 25	1.0

12. Keywords

12.1 gaseous fuels

ATTACHMENT 7

Utah State
UNIVERSITY

DEPARTMENT OF NUTRITION AND FOOD SCIENCES
College of Agriculture
College of Family Life
Logan, UT 84322-8700
Telephone: (435) 797-2126
FAX: (435) 797-2379

Division of GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St, SW
Washington, DC 20204

August 17, 2001

Dear FDA Personnel,

I am a meat scientist with experience in studying the effects on fresh meats of various modified atmospheres. Based on my review of the details of the ActiveTech 2001 modified atmosphere system employing 0.4% carbon monoxide gas in a mixture with 60 percent carbon dioxide and the remainder nitrogen, as well as the published literature and common knowledge in the field, I confirm that the use of modified atmospheres including 0.4% CO to package fresh meats as used in the ActiveTech 2001 system is both safe and generally recognized as safe. If I can provide any further information or clarification, please contact me.

Sincerely yours,

Daren Cornforth

Daren Cornforth, Ph.D.
Professor, Nutrition & Food Sciences
Utah State University
435-797-2114
darenc@cc.usu.edu

Daren Cornforth, Ph.D.

Birthdate: May 23, 1949 Birthplace: Fort Collins, Colorado

Current Address: Department of Nutrition and Food Sciences
Utah State University
Logan, Utah 84322-8700
(435) 797-2114 fax (435) 797-2379
e-mail darenc@cc.usu.edu

Education

Institution	Degree	Year Conferred	Scientific Field
Colorado State University	B.S.	1971	Animal Science
Colorado State University	M.S.	1973	Animal Science
Michigan State University	Ph.D.	1978	Food Science & Human Nutrition

Research and Professional Experience

Dates	Position	Duties
1978 to present	Asst., Assoc., Full Prof., NFS	Teaching/Research, Meat Processing
1974 to 1978	Graduate Research Assistant at Michigan State University East Lansing, Michigan	Dissertation on cold- induced shortening and toughening of beef muscle.
1971 to 1973	Graduate Research Assistant at Colorado State University Fort Collins, Colorado	Thesis on the effects of breed, sex, and diet on muscle fiber type and fat cell development in growing calves.

Memberships

Institute of Food Technologists, Amer. Assoc. for the Advancement of Science, Sigma Xi (Past President, USU Chapter), American Meat Science Association, Farm House Fraternity, Rocky Mountain Elk Foundation (Life Member), Pheasants Forever, Nature Conservancy, Cache Valley Wildlife Federation (and on Executive Board).

Current Teaching Assignment

NFS 5560 Chemistry of Food Systems
NFS 6450 Meat Science
NFS 1000 World of Food and Nutrition

I also assist with the NFS Food Science Club College Bowl team, and coordinate (with Dr. Carpenter) the Utah Future Farmers of America (FFA) state contest in Food Science and Technology.

Invited Speaker (1997-2001)

Comforth, D. P. 1997. *Pigment concentration and pH effects on degree of doneness of beef patties.* FMC Corporation. Atlanta, Ga, January, 1997.

Comforth, D. P. 1997. *Nitrogen dioxide causes surface pinking on meats cooked in gas ovens.* Meat Industry Meat Conf. Chicago, IL, Oct 28-29.

Comforth, D. P., Jiang, C. and Mumford, B. 1998. *Effect of storage time, storage and cooking temperature, and holding time after cooking on pigment levels in beef patties.* Symposium, New Developments in Meat Processing, Amer. Meat Sci. Assn. National Meeting, Storrs, CT., June 28-July 2.

Comforth, D. P. 2000. Keynote speaker. *Pinking in Cooked Poultry. Current situation and general theories.* Given at the Symposium on Pinking in Cooked Poulty, Center for Excellence in Poultry Science, University of Arkansas, Fayetteville, Oct 12-13, 1999. Co-sponsored by Tyson Foods, NewlyWed Foods, and KFC, Inc.

Comforth, D. P. 2000. *Pinking in Cooked Poultry.* Michigan State University, E. Lansing, MI, Nov. 7.

Comforth, D. P. 2000. *Pinking in Cooked Poultry.* Michigan Turkey Processors, Zeeland, MI, Nov. 8.

Comforth, D. P. 2001. *Meat Color.* Dinner speaker for the joint meeting of the Southern Minnesota section of IFT and the Meat Processing Short Course sponsored by the Agricultural Utilization Research Institute, Southwest State University, Marshall, MN, April 25.

National Offices and Committees (1997-2001)

USDA Advisory Committee for the Safe Preparation of Ground Beef - 1997-8.

AMSA (American Meat Science Association)
Graduate Student Poster Competition committee, Reciprocal Meat Conference, 1998-2002.

Executive Board 2000-2002.

IFT Executive Committee, Muscle Foods Division, Institute of Food Technologists, 1998-99.

Chair-Elect, Muscle Foods Division, 2000-2001.

Chair, Muscle Foods Division, 2001-2002.

Executive Council, Bonneville Section, IFT (Councilor representing Bonneville Section at National Meetings), 1998-99.

Journal of Muscle Foods – Editorial Board, 1998-2002

University Committee Assignments

Committee for Laboratory Safety. 1997-2001.
Faculty Senate, 2000 – 2003.
Faculty Senate Executive Committee 2000 – 2001.

Grants (1997-2001)

Cornforth, D. P. 1997-98. Factors affecting hamburger degree of doneness. McDonald's Corp. Oak Brook, IL. $8,480.

Cornforth, D. P. 1998. Evaluation of various dairy fractions as inhibitors of lipid oxidation in precooked meats. Western Dairy Foods Center, Logan, UT, $9,560.

Cornforth, D. P. 1999. Verification of nutritent label claims on lowfat milk, lean hamburger, and breakfast cereals. Dr. Alan Luke (Alumni Donor), $1,500.

Cornforth, D. P. and Carpenter, C. E. 1999-2000. Evaluation of carbon monoxide treatment in modified atmosphere or vacuum packaging to increase color stability of fresh beef. National Cattlemen's Beef Association, $14,500.

Carpenter, C.E. and Cornforth, D. P. 1999-2000. Effect of consumer bias for beef color and packaging on eating satisfaction. National Cattlemen's Beef Association, $15,000.

Cornforth, D. P. 1999-2002. Evaluation of antioxidant properties of milk powders in cooked meats. Glanbia Foods, Twin Falls, ID. $30,000.

Cornforth, D. P. 2000-2002. New antioxidants in cooked meats. Utah Agric. Exp. Stat. $15,000.

Bailey, D. and Dickinson, D. (Cornforth, D.P., co-investigator). 2001-2003. Traceability: A market opportunity or threat to the US meat industry. USDA-CREES. $160,000.

Cornforth, D. P. 2001-2002. Dried whey minerals as an antioxidant in processed meats. Dairy Management Institute (DMI). $97,500.

Graduate Committees Chaired (1997-2001)

Heaton, K. M. 1998. Minimum levels of nitrite, nitric oxide, and carbon monoxide causing pinking in cooked beef and turkey rolls. M. S. thesis, Utah State University, Logan, Utah. (Now Extension Agent, Garfield & Kane Counties, Utah).

Moiseev, I. V. 1998. Prevention of pink discoloration and microbial safety of rolls and patties made from dark-cutting beef, PhD dissertation, Utah State University, Logan, Utah. (Now lab chemist, Borden Foods, Columbus, OH).

Racz, J. M. 1998. An inoculated pack study using *Clostridium sporogenes* PA 3679 to determine the shelf stability of a vacuum packaged meat/vegetable stick. M. S. thesis, Utah State University, Logan, Utah. (Now lab chemist, Fresenius Medical, Ogden, UT).

Jayasingh, P. 2001. Dried milk minerals as an antioxidant in processed meats. Ph.D. dissertation, Utah State University, Logan, Utah (work in progress).

Product Development and Extension

Stew Sticks are currently in retail production by Utah Jerky, Inc., Ogden, UT.

Cornforth, D. P., Bailey, D., and McEvoy, R. 1997. Developed a video of ostrich slaughter and processing, used by the new plant in Filmore, UT for employee training, and also used by USU extension agents.

Refereed Publications (1997-2001)

Moiseev, I. V. and Cornforth, D. P. 1997. Sodium hydroxide and sodium tripolyphosphate effects on bind strength and sensory characteristics of restructured beef rolls. Meat Sci. 45:53-60.

Quinton, R. D., Cornforth, D. P., Hendricks, D. G., Brennand, C. P. and Su, Y. K. 1997. Acceptability and composition of some acidified meat and vegetable stick products. J. Food Sci. 62:1250-1254.

Cornforth, D. P., Rabovitser, J. K., Ahuja, S., Wagner, J. C., Hanson, R., Cummings, B. and Chudnovsky, Y. 1998. Carbon monoxide, nitric oxide, and nitrogen dioxide levels in gas ovens related to surface pinking of cooked beef and turkey. J. Agric. Food Chem. 46:255-61.

Lee, B., Hendricks, D. G. and Cornforth, D. P. 1998. Antioxidant effects of carnosine and phytic acid in a model beef system. J. Food Sci. 63:394-398.

Lee, B., Hendricks, D. G. and Cornforth, D. P. 1998. Effect of sodium phytate, sodium pyrophosphate, and sodium tripolyphosphate on physicochemical characteristics of restructured beef. Meat Sci. 50:273-283.

Moiseev, I. V. and Cornforth, D. P. 1999. Treatments for prevention of persistent pinking in dark-cutting beef patties. J. Food Sci. 64:738-43.

Lee, B., Hendricks, D. G. and Cornforth, D. P. 1999. A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef pattie model system. Meat Sci. 51:245-253.

Heaton, K. M., Cornforth, D. P., Moiseev, I. V., Egbert, W. R. and Carpenter, C. E. 2000. Minimum sodium nitrite levels for pinking of various cooked meats as related to use of direct or indirect-dried soy isolates in poultry rolls. Meat Sci. 55:321-329.

Carpenter, C. E., Cornforth, D. P., Whittier, D. 2001. Consumer preferences for beef color and packaging did not affect eating satisfaction. Meat Science 57:359-363.

Jayasingh, P. Cornforth, D. P., Carpenter, C. E., and Whittier, D. 2001. Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. Meat Science, In press.

Book Chapters

Cornforth, D. P. 2000. **Miscellaneous Colorants - Cured Meat.** Unit F6.2 in *Current Protocols in Food Analytical Chemistry.* S. J. Schwartz, Ed. John Wiley & Sons, Inc. New York, NY.

Cornforth, D. P. 2001. **Potential use of phytate as an antioxidant in cooked meats.** Ch. 11 in *Food Phytates.* R. Reddy and S. K. Sathe, Eds. Technomic Publ., Inc., Rowayton, CT.

ENVIRON

August 27, 2001

Eric Greenberg
Ungaretti & Harris
3500 Three First National Plaza
Chicago, IL 60602-4283

Re: Generally recognized as safe ("GRAS") determination for carbon monoxide from Pactiv ActiveTech food-contact packaging.

Dear Eric:

This letter reports my assessment, based on my review of the available information, that the potential consumer exposure to carbon monoxide from the Pactiv ActiveTech food-contact packaging is safe, and is also generally recognized as safe ("GRAS"), under the Federal Food, Drug, and Cosmetic Act ("FDCA" or "the Act") when used under conditions defined in the attached GRAS determination document. First, the safety of carbon monoxide in this use is demonstrated by comparison of the U.S. Environmental Protection Agency ("USEPA") national ambient air quality standard ("NAAQS") for carbon monoxide with the estimated daily intake ("EDI") of carbon monoxide under the intended conditions of use of the product. Exposure to a substance generally is considered safe for its intended use if the EDI is a fraction of the allowable daily intake ("ADI"). Second, the GRAS status of carbon monoxide in this use is affirmed by demonstrating that the safety of carbon monoxide from Pactiv ActiveTech food-contact packaging material in its intended use is generally recognized.

My GRAS determination was based on scientific procedures as outlined in the U.S. Food and Drug Administration ("FDA") regulations (at §170.30(b)). This section requires that the same quantity and quality of scientific evidence is available and is reviewed as is required to obtain approval of the substance as a food additive. Moreover, in addition to requiring scientific evidence of safety (as with a food additive), a GRAS determination also requires that this scientific evidence of safety be generally known and accepted by experts qualified in the appropriate scientific and technical fields. This common knowledge requirement of a GRAS determination includes two elements: (1) the data and information relied upon to establish the scientific basis for safety must be generally available; and (2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

Based on the scientific literature, studies, conclusions, and restrictions presented in the GRAS determination document, I regard the proposed uses of carbon monoxide from Pactiv ActiveTech packaging material as generally recognized as safe because the use will result in an exposure that is well below an acceptable exposure level for carbon monoxide.

ENVIRON International Corporation
4350 North Fairfax Drive, Suite 300 • Arlington, VA 22203 USA
Tel: (+1) (703) 516 2300 • Fax: (+1) (703) 516 2393 • www.environcorp.com

No evidence exists in the available information on carbon monoxide that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when carbon monoxide is used at levels that might reasonably be expected from its proposed use as an additive in the Pactiv ActiveTech food-contact packaging material as defined in the GRAS determination document.

It is my opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, carbon monoxide, as an additive in the Pactiv ActiveTech food-contact packaging material, is generally recognized as safe.

Vasilios Frankos

Vasilios H. Frankos, Ph.D.
Principal, Health Sciences
ENVIRON International Corporation
Arlington, Virginia

VASILIOS H. FRANKOS, Ph.D.

EDUCATION

1977 Ph.D., Pharmacology and Toxicology, University of Maryland Pharmacy School

1973 M.S., Biology, University of Maryland

1970 B.A., Biology, University of Maryland

EXPERIENCE

Dr. Frankos is a Principal at ENVIRON Corporation and has over 20 years of experience in the toxicological and pharmacological evaluation of data used to assess the risks posed by foods and food additives, drugs, medical devices, cosmetics, pesticides, and environmental and occupational exposures. He has also been involved in the development of exposure and risk assessment methodology. Since joining ENVIRON, Dr. Frankos has led, contributed to, or managed hundreds of projects in these areas.

Foods and Food Additives:

Dr. Frankos has worked on a wide variety of projects evaluating the safety of foods, direct and indirect food additives, and food contaminants. As part of these food-related safety evaluations, he has developed strategies for testing new direct and indirect additives, evaluated toxicity test data to support safety determinations, prepared Generally Recognized as Safe (GRAS) reviews, performed exposure and risk assessments, and developed regulatory strategies. Dr. Frankos has also presented safety evaluations to the FDA on behalf of clients. Some of his major projects in the area of foods and food additives include:

- Provided ongoing FDA-related scientific and technical support to the Coalition for Safe Ceramicware (CSC). Performed a safety assessment of ceramic pitchers with glazes containing lead using data collected on migration of lead into a food simulant and into real foods. This assessment was submitted to the FDA as part of the CSC's comments on the FDA's proposed rule changing the action level for lead from ceramic pitchers.
- Developed direct food additive petitions and GRAS self-affirmation documents for numerous food additives including, novel fibers sources, an anti-caking agent, enzymes, sugars, and a major new class of food additives.
- Conducted a GRAS self-affirmation review, including an evaluation of safety data, of the first bioengineered food approved by the FDA. Presented this GRAS review to the FDA on behalf of the client, a major biotechnology company.
- Developed a GRAS affirmation document for a cellulose product, manufactured by a novel bacterial fermentation process, with proposed food use as a suspending/thickening agent. Designed, placed, and monitored preclinical toxicity studies required for FDA approval.
- Estimated doses posing no significant risk for chemicals that could potentially leach from packaging into food. Assessed the potential human exposure to these chemicals from migration from packaging into food. Compared the potential ingested dose to the no significant risk dose.

- Petitioned the FDA to sanction expanded use in foods of an approved food additive. Prepared a review and update of existing toxicological literature on the material and estimated the increase in exposure likely to result from proposed new uses.
- Evaluated the carcinogenic risk associated with exposure to acrylamide residues in food and methylene chloride in decaffeinated tea.
- Addressed issues relating to FDA's regulation of polychlorinated biphenyl (PCB) residues. Examined whether tolerances for PCBs in fish could be reinterpreted for less chlorinated PCBs that have lower or no carcinogenic potency. Determined necessary research to establish differences in potency between PCBs.
- Developed an innovative exposure and safety assessment for a novel single cell protein (mycoprotein) meat substitute that has been submitted to the FDA for approval.
- Conducted a simulated FDA review of a food additive petition on a new artificial sweetener submitted to the FDA by the client's competitor. Review included critical evaluation of product chemistry, efficacy, estimates of human exposure, animal and human toxicology data, pharmacokinetics and metabolism information, and the basis for determining the acceptable daily intake of the sweetener.
- Performed a detailed evaluation of toxicity and carcinogenicity studies sponsored by a major drug company and studies from the published literature for the company's non-nutritive sweetener and assessed the toxicological significance to humans. Assisted in submission of a food additive petition. Provided continued regulatory support during the FDA review process.

Human and Veterinary Drugs, Medical Devices, and Cosmetics:

Dr. Frankos has provided scientific and regulatory guidance to clients in the human and veterinary drug, medical device, and cosmetic industries. He has been involved in identifying and assessing the risks to humans associated with exposure to constituents of these products. He has assisted clients in these industries in interacting with the FDA and has assisted them in complying with all aspects of FDA regulations. Some of his major projects in the areas of drugs, medical devices, and cosmetics include:

- Reviewed two large epidemiological studies on the differences in adverse drug reaction rates between two types of radiographic contrast media. Prepared a safety review document on animal and human literature on contrast media.
- Performed an independent evaluation of a New Drug Application (NDA) submission to the FDA, with emphasis on review of efficacy studies.
- Assisted the medical device manufacturer in complying with FDA's post-approval requirements for its device including compliance with the Medical Device Reporting (MDR) rule, submission of updates to the PMA application, and ensuring that all labeling and marketing materials are in compliance with FDA regulations. Designed a post-marketing clinical trial for the device to comply with FDA recommendations.
- Evaluated the potential carcinogenic risks to humans of an over-the-counter (OTC) medication that is applied to the skin. Prepared a report on these findings that was submitted to the FDA.

VASILIOS H. FRANKOS, Ph.D.

- Prepared and submitted to the FDA a New Drug Application (NDA) for a drug that holds promise for dramatically decreasing the high percentage of reocclusion that occurs in angioplasty patients.
- Assisted a major pharmaceutical manufacturer in assessing potential health risks associated with a specific ingredient of various over-the-counter (OTC) drugs.
- Critically evaluated both published and unpublished studies on a psychoactive drug and rendered an opinion to the client on potential health effects of the drug and whether a no-observed-effect level (NOEL) had been established.
- Provided guidelines for subchronic testing to evaluate the safety for human use of an allergen desensitizer that was produced by polymerizing the allergen through a glutaraldehyde treatment.
- Analyzed the potential risk to humans resulting from the use of Furazolidone as an animal drug. Determined an estimate of this risk and presented the estimate to the FDA at a public hearing.
- Assisted a major manufacturer of veterinary drug products in developing an approach to dealing with mouse liver tumors and their usefulness as evidence of carcinogenicity.
- Reviewed and evaluated a New Animal Drug Application for FDA submission. Advised on the necessity of future studies.
- Assembled an expert panel to address the safety of an antimicrobial agent, extracted from a plant source, for use in oral hygiene products (e.g. toothpaste and oral rinse). The evaluation included a review of the preclinical and clinical toxicologic database, analysis of exposure, and determination of margin of safety associated with the proposed oral uses.
- Critically evaluated the evidence cited by FDA as the basis for considering nitrofuran animal drugs to be carcinogenic under the meaning of the Delaney Clause of the Federal Food, Drug, and Cosmetic Act.
- Reviewed toxicity data to be submitted in support of an Investigational Device Exemption (IDE) for an implantable medical device. Recommended and monitored the performance of supplemental tests, performed an exposure assessment of substances leaching from the device into the systemic circulation, characterized the risk to health from such exposures, and assisted in the presentation of these findings to the FDA.
- Evaluated toxicity data on the materials used in a device intended to be implanted in the abdominal cavity. Examined the adequacy of the existing data on the device material, the safety of the material used, and the safety of the proposed replacement material. Recommended studies to improve the data for submission to the FDA.
- Conducted a quantitative risk assessment on numerous color additives used in dermally applied cosmetics including an evaluation of toxicity, an analysis of exposure, and a determination of quantitative risks.
- Performed a hypothetical risk assessment for two colors used in cosmetics, based on the assumption that, if tested, they would produce tumors in rats. Demonstrated that such an outcome would still allow continued safe use of these colors in cosmetics.

Pesticides:

Dr. Frankos has assisted U.S. and foreign manufacturers in obtaining EPA and California registration of agricultural, forestry, and homeowner use pesticide products. Some of his major projects in the area of pesticides include:

Reviewed EPA's assessment of dietary oncogenic risk of two fungicides and advised the manufacturers of additional data needed to perform a quantitative risk assessment.

- Designed and supervised a field study to estimate exposure to a pesticide during "worst case" application. The study monitored the application of the chemical, measured exposure of the user during various phases of application and determined the effect of protective clothing on exposure.
- Reviewed the results of an aquatic organism field monitoring study and its supporting laboratory data for a major manufacturer of agricultural chemicals.
- Evaluated toxicity and prepared a risk assessment for residues of a fungicide in imported wines. Counseled client on process necessary to receive an EPA import tolerance for the fungicide. Advised client on additional data needed to support a tolerance.
- Designed and monitored toxicology studies for a German firm required for registration of a plant growth promotor and assisted in submitting data to the EPA.
- For a West German pesticide manufacturing company wishing to purchase the patent rights to a new pesticide developed in the U.S., provided counsel on the acceptability of the available data to EPA and OECD and the further data needed to obtain a registration in the U.S.

Environmental and Occupational Exposures:

Dr. Frankos has directed numerous exposure and risk assessments involving hundreds of chemicals that have been associated with industrial processes, toxic waste or municipal incinerators, and hazardous waste sites. These assessments have used computerized models and include all routes of exposure. Some of his major projects in the areas of environmental and occupational exposures include:

- Performed a safety assessment for the consumption of drinking water in contact with a piece of equipment that could potentially release lead, including an estimation of 1990 baseline blood lead levels for four subpopulations using the Integrated Uptake/Biokinetic Model and a determination of the maximum acceptable concentration of lead in drinking water that was potentially in contact with the equipment. Collected and reviewed information on factors that affect the leachability of lead into drinking water.
- Assisted manufacturers of the plasticizer di-(2-ethylhexyl) phthalate (DEHP) in developing a method for estimating exposure resulting from the chemical's presence in polyvinyl chloride (PVC) consumer products such as vinyl-covered furniture, vinyl wallpaper, flooring, and shower curtains.
- Critically reviewed acrylamide's carcinogenic activity in Fischer 344 rats and designed a new cancer bioassay in rats that will improve risk assessments for acrylamide.

- Reviewed the scientific basis for establishing safety factors needed to protect workers from reproductive toxicity associated with glycol ether exposure.
- Reevaluated the data from a National Cancer Institute (NCI) bioassay on dibutyltin diacetate to resolve discrepant interpretations by NCI and FDA.
- Reviewed FDA and National Toxicology Program (NTP) evaluations of the bioassay on dimethylterephthalate and rendered an opinion on the adequacy of the NTP and FDA decisions.
- Supported the EPA Office of Solid Waste in developing measures of inherent toxicity which can be combined with exposure estimates to provide a relative ranking for scheduling hazardous solid waste.
- Provided scientific litigation support for a municipality in which its waste treatment plant and landfill were contaminated with PCBs.
- Participated in a number of projects examining the developmental, embryotoxic, and teratogenic effects of lead observed in animals, including the effects of lead on the reproductive systems of male and female rats. Recommended a no-observed-adverse-effect level (NOAEL) for lead.

Exposure and Risk Assessment Methodology Development:

Dr. Frankos has participated in the development of new exposure and risk assessment methodologies for federal and state regulatory agencies. He has also been integral in the development of exposure and risk modeling software. Some of his major projects in these areas include:

- Developed a background document for the EPA on reproductive toxicity risk assessment for use in drafting interagency risk assessment guidelines.
- Directed development of a scheme for the EPA that allows severity of toxic effects to be incorporated into safety evaluations of EPA regulated products.
- Summarized and compiled comments received by EPA on their proposed guidelines (1985) for developmental toxicity assessment.
- Assisted in evaluating EPA's procedures for estimating safe short-term exposure limits and in developing an alternative method that could be uniformly used by the entire agency.
- Assisted in the development of criteria for listing chemicals as developmental toxicants under California's Proposition 65.
- Directed the development of a computer software system, ERMA (Exposure and Risk Modeling Assistant) that enables ENVIRON to provide high quality, scientifically defensible evaluations of potential exposures and resultant risk.

Before joining ENVIRON Corporation, Dr. Frankos held the following positions:

- Associate Director, Life Sciences Division, Clement Associates. In that position he had the following experience:
  - Supervised a staff of eight scientists who assessed the risk posed by environmental contaminants, occupational carcinogens, pesticides, drugs, commercial product

constituents, and food additives. Many of these projects involved evaluating human and animal toxicology data for use in conducting human risk assessments. This position required management of time constraints, budgetary limitations, and personnel allocations in a manner that provided the client with a scientifically defensible document.

- Directed preparation of reports for industry clients that included "Safety Assessment Strategies for Feminine Hygiene Products"; "The Impact of a Proposed Salicylate Warning on the Risk Associated with Diseases and Conditions in Children"; and "A Proposed Mechanism of Action for a Carcinogenic Hair Dye Ingredient."
- Directed and prepared a report to OSHA entitled "Formaldehyde Risk Assessment for Occupationally Exposed Workers" and assisted in developing guidelines for interspecies data extrapolation for use by OSHA in its revised cancer policy.
- Provided litigation toxicity support to private and industry clients and assisted private concerns in the development of testing protocols for the purposes of fulfilling regulatory requirements.
- Served as expert reproductive toxicity witness in the House of Representative's hearing on the "Relationships of Exposure to Toxic Chemicals and Reproductive Impairment."

• Staff Science Advisor, Office of Health Affairs, Commissioners Office, Food and Drug Administration.

- Provided professional scientific expertise in pharmacology, toxicology, biochemistry, and biology requisite to the effective accomplishment of the Agency's scientific overview and leadership function. Served as technical expert, scientific advisor, and liaison of the Commissioner at the Bureau level on matters relevant to toxicologic and pharmacologic safety assessment of toxic substances to which humans and animals are exposed.
- Responsibilities included performing risk assessments on compounds (such as PCB's, methapyraline, caffeine, etc.) that could be utilized by the Commissioner's office in choosing between various regulatory options. Routinely reviewed toxicity data on compounds as diverse as caffeine, methylsalicylate, xylitol, and sodium nitrite. Provided litigation support and testified as expert toxicology witness at the Administrative Law Judge hearing on the safety of cyclamate.
- Participated in the National Toxicology Program (NTP). Duties included review of agency nominations for toxicity testing by NTP, research planning for the regulatory needs of FDA, preparation of the Annual Carcinogen Report, and participation in numerous NTP workgroups.
- Interagency and international initiatives included chairing the Interagency Regulatory Liaison Group (IRLG) Workgroup on Reproductive Toxicity Risk Assessment. Planned, coordinated, and published a three-day symposium entitled, "The Effects of Foods and Drugs on the Development and Function of the Nervous System." Chaired and organized a national workshop on Reproductive Toxicity Risk Assessment.
- Served as Project Director of a contract with the Environment Teratology Information Computer Division, Oak Ridge National Research Laboratory to develop computerized teratology literature data extraction, indexing, and collation. This data base system is being

integrated into the ETIC master computer file and will be manipulated through the INQUIRE data base management system.

- Senior Toxicologist, Division of Toxicology, Center for Food Safety and Applied Nutrition, Food and Drug Administration.
  - Responsible for the toxicologic evaluation of compounds of special interest to the agency. Required expert review of data on carcinogenicity, promotion, reproduction, teratology, and pharmacokinetics. Presented scientific evaluations to congressional, departmental, and agency directors. Represented the agency on sensitive scientific issues. Areas of emphasis included evaluation of toxicity data on artificial sweeteners (saccharin, cyclamate, xylitol, etc.), development of reproductive toxicity risk assessment criteria, pharmacokinetics evaluation of compounds (methylene chloride, sodium nitrite, saccharin, etc.), and risk assessment.
  - Directed the development of a PC computer network to be used by the Review Toxicologist of the FDA Bureau of Foods. This system allows direct access to toxicology data bases at NLM, Oak Ridge, Dialog, and extensive FDA internal data bases and full manipulation, storage, and collation of personalized literature data bases.
- Toxicologist, Division of Toxicology, Bureau of Foods, Food and Drug Administration.
  - Responsible for toxicologic review of substances used as direct and indirect food additives. Duties required discussions with manufacturers, formulators, toxicologists, universities, and other agencies on experimental procedures for showing safety and estimating risk.
  - Additional duties and accomplishments included primary involvement in planning, testing, and implementing new proposed regulations that direct the priority assessment review of all food additives. Duties included designing and implementing modern toxicity testing protocols, protocol quality parameters, criteria for utilizing toxicity data, and computer compatible toxicology test summarization forms. Co-led a 12 person toxicology cyclic review team. This endeavor was awarded a commendable service citation.
  - Served as the toxicology member on the Program Advisory Board for the FDA's effort to modernize the storage and retrieval of safety information in the Bureau of Foods. Over two million dollars was expended for the computerization and microfiching of all Bureau petition files. This system is now fully operational within the FDA and is called SIREN.
- Research Assistant, Department of Pharmacology and Drug Abuse, Maryland Psychiatric Research Center.
  - Laboratory responsibilities included the daily analysis of the catecholamines and their metabolites in the urine of patients under different drug therapies. Used column chromatography to separate the monoamines and their metabolites with subsequent fluorometric determinations of their amounts. Techniques involved using radioisotopic methods to determine monoamine oxidase kinetics, substrate km, and oxygen requirements and preparing mitochondrial isolates to study by oxygraphic assay and radioisotopic methods.
  - Clinical responsibilities included the collection, collation, and statistical analysis of data obtained by researchers in various disciplines, such as clinical and physiological psychology, biochemistry, and pharmacology. Assisted in the design, implementation,

and statistical analysis of a study concerning the use of naloxone and cyclazocine as narcotic antagonists in a population of paroled drug addicts. Implementation of the study involved administering, scoring, and statistically analyzing psychodiagnostic and intelligence tests.

HONORS

1980 FDA Commendable Service Award for Direct Food Additive Cyclic Review

1969-70 Baltimore University Club Scholarship

1966-67 American Hellenic Education Scholarship

PROFESSIONAL ACTIVITIES AND MEMBERSHIPS

Invited presentation entitled "Developmental and Reproductive Toxicity Testing of Implantable Medical Devices." Presentation for: 1996 Summer Short Course Series on Medical Device Biocompatibility: From Material Screening to Final Product Testing. Sponsored by: Case Western Reserve University. July 18-19, 1996, Baltimore, Maryland.

Invited presentation entitled "Risk Assessment: What is it? How can I use it?" Regional Meeting of the Association of Official Analytical Chemists (AOAC), October 27, 1994, College Park, Maryland.

Invited presentation entitled "Testing Requirements for Medical Devices" Annual Genetic Toxicology Workshop, May 3-5, 1993, Rockville, Maryland.

Society of Regulatory Toxicology and Pharmacology 1990 -

Food and Drug Law Institute 1990 -

Guest Lecturer, University of Vermont Law School "Risk Assessment at the Law" June 4-14, 1990, Burlington, Vermont.

Invited presentation entitled "Review of Safety and Toxicity of Sanguinaria and Sanguinarine" Symposium on Sanguinaria, April 25, 1990, Toronto, Ontario, Canada.

Invited presentation entitled "Health Risks and Safety Precautions" PCB Compliance, Cleanup and Disposal, March 27-28, 1990, Toronto, Ontario, Canada.

Environmental Law Institute 1989 -

Invited panel member "Weight of Evidence Considerations in Identifying Reproductive and Developmental Toxicants" Risk Assessment Issues in Developmental and Reproductive Toxicology, Sept. 18-19, 1989, Berkeley, California.

Invited presentation entitled "Health Risk and Safety Precautions" Current Issues in PCB Compliance, May 24-25, 1989, Toronto, Canada.

Regulatory Affairs Professionals Society 1988 -

Invited presentation entitled "Risk Assessment for Effects Other than Cancer" 1986 Conference for Food Protection, Aug. 17-20, 1986, Ann Arbor, Michigan.

Invited work group member at the EPA sponsored "Consensus Workshop on the Evaluation of Maternal and Developmental Toxicity" May 12-14, 1986, Rockville, Maryland.

Co-author of an invited paper entitled "Acrylonitrile as a Carcinogen: Research Needs for Better Risk Assessment" presented at the International Conference on "Occupational and Environmental Significance of Industrial Carcinogens" October 7-9, 1985, Milan, Italy.

Invited presentation entitled "FDA Perspectives on the use of Teratology Data for Human Risk Assessment" Symposium on "Risk Assessment for Developmental Toxicity" Annual meeting of the Society of Toxicology, March 13, 1984, Atlanta, Georgia.

Guest Lecturer and Expert Consultant to the Environmental Teratology Information Computer Division, Oak Ridge National Research Laboratory. 1982.

Expert Witness before the U.S. House of Representatives Committee on Science and Technology hearing on the "Relationship of Exposure to Toxic Chemicals and Reproductive Impairment," 1982.

Guest speaker, FDA National Scientific Health Professionals Meeting; spoke on the National Toxicology Program. 1981.

Member, National Toxicology Program Chemical Evaluation Committee. 1979-1982.

Member, National Toxicology Program Annual Carcinogen Report Workgroup. 1979-1982.

Invited workshop panel member, Workshop on Biological and Statistical Implications of the ED Study on Related Data Bases, Mt. Sterling, Ohio; sponsored by the Society of Toxicology for the National Center for Toxicological Research. 1981.

Chairman - Interagency Regulatory Liaison Group - Reproductive Toxicity Risk Assessment Group. 1980-1981.

Chairman and Organizer of the IRLG - Reproductive Toxicity Risk Assessment Workshop held at FDA, Rockville, MD. 1981.

Participated in preparation of the National Science Foundation Third Annual Science and Technology Report. 1980.

Participated in Preparation of the National Science Foundation Five-Year Science Outlook. 1980.

Participated in publication of the First Annual Report on Carcinogens (DHHS/NTP). 1980.

Organizer and lecturer for a State Department and DHHS/FDA sponsored course conducted for the National Organization for Drug Control and Research (Cairo, Egypt), entitled: "A Course in Chronic and Reproductive Toxicity Testing and Risk Assessment." 1980.

Guest lecturer at FDA Consumer Exchange Meeting: Use of Risk Assessment for Decision-Making. 1980.

Co-chairman and organizer of the Fifth FDA Science Symposium on Effects of Foods and Drugs on the Development and Function of the Nervous System: Methods for Predicting Toxicity. Delivered presentation entitled "Symposium Summary and Future Directions for Neurotoxicology Testing." 1979.

Expert witness FDA Administrative Law Judge hearing on the Safety of Cyclamate Used as an Artificial Sweetener. 1979.

PUBLICATIONS

Kruger, C.L., M.H. Whittaker, and V.H. Frankos. 1999. Genotoxicity Tests on D-Tagatose. *Regulatory Toxicology and Pharmacology*. 29(2):S36-S42.

Kruger, C.L., M.H. Whittaker, and V.H.Frankos. 1999.Developmental Toxicity of D-Tagatose. *Regulatory Toxicology and Pharmacology*. 29(2):S29-S35.

Turnbull, D., M.H. Whittaker, V.H. Frankos, and D. Jonker. 1999. 90-Day Oral Toxicity Study of D-Tagatose in Rats. *Regulatory Toxicology and Pharmacology*. 29(2):S1-S10.

Trunbull, D., M.H. Whitaker, V.H. Frankos, and D. Jonker. 1999. 13-Week Oral Toxicity Study with Stanol Esters in Rats. *Regulatory Toxicology and Pharmacology*. 29(1):216-226.

Whittaker, M.H., V.H. Frankos, D.H. Waalkens-Berendsen, A.P.M. Wolterbeek. 1999. 2-Generation Reproductive Toxicity Study of Plant Stanol Esters in Rats. *Regulatory Toxicology and Pharmacology*. 29(1):196-204.

Hester, T.R., N.F. Ford, P.J. Gale, J.L. Hammett, R. Raymond, D. Turnbull, V.H. Frankos, and M.B. Cohen. 1997. Measurement of 2,4-toluenediamine in urine and serum samples from women with même or replicon breast implants. *Plastic and Reconstructive Surgery*. 100(5):1296-1298.

Silverstein, B., K.M. Witkin, V.H. Frankos, and A.I. Terr. 1997. Assessing the Role of the Biomaterial Aquavene in Patient Reactions to Landmark Midline Catheters. *Regulatory Toxicology and Pharmacology.* 25(1).

Rodricks, J.V., V.H. Frankos, and L.M. Plunkett. 1995. Food Additives. In: Regulatory Toxicology. C.P. Chengeliss, J.F. Holson and S.C. Gad (eds.) Raven Press, New York, New York, 51-82.

Spiegel, J.E., R. Rose, P. Karabell, V.H. Frankos, and D.F. Schmitt. 1994. Safety and Benefits of Fructooligosaccharides as Food Ingredients. *Food Technology.* 85-89.

Redenbaugh, K., T. Berner, D. Emlay, V. Frankos, W. Hiatt, C. Houck, M. Kramer, L. Malyj, B. Martineau, N. Rachman, L. Rudenko, R. Sanders, R. Sheehy, and R. Wixtrom. 1993. Regulatory Issues for Commercialization of Tomatoes with an Antisense Polygalacturonase Gene. *In Vitro Cell. Dev. Biol.* 29:17-26.

Frankos, V.H., D.F. Schmitt, L.C. Haws, A.J. McEvily, R. Iyengar, S.A. Miller, I.C. Munro, F.M. Clydesdale, A.L. Forbes, and R.M. Sauer. 1991. Generally Recognized as Safe (GRAS) Evaluation of 4-Hexylresorcinol for Use as a Processing Aid for Prevention of Melanosis in Shrimp. *Regulatory Toxicology and Pharmacology* 14:202-212.

Wilcock, K.E., A.B. Santamaria, V.H. Frankos, H.W. Fischer, F. Laden, E.A. Platz, and B.A. Jackson. 1990. Perspectives on Adverse Reaction Rates Associated with the Use of High Osmolar Ionic and Low Osmolar Nonionic Contrast Media. *Journal of the American College of Toxicology* 9(6):563-607.

Frankos, V.H., D.J. Brusick, E.M. Johnson, H.I. Maibach, I. Munro, R.A. Squire, and C.S. Weil. 1990. Safety of Sanguinaria Extract as Used in Commercial Toothpaste and Oral Rinse Products. *Journal of the Canadian Dental Association* 56(7 Suppl):41-47.

Schmitt, D., V. Frankos, and D. Richardson. 1990. Toxicologic Evaluation of Sanguinaria Extract. Eleventh Annual Meeting of the American College of Toxicology. Program and Abstracts. Abstract.

Schmitt, D., V. Frankos, J. Westland, and T. Zoetis. 1990. Toxicological Evaluation of Cellulon™ Fiber: Genotoxicity, Acute and Subchronic Toxicity. Eleventh Annual Meeting of the American College of Toxicology. Program and Abstracts. Abstract.

Rudenko, L., J. Adgate, M. Aponte-Pons, T. Berner, V. Frankos, R. Gregory, C.K. Lintner, N. Rachman, W. Sherman, T. Winters, and R. Wixtrom. 1990. Application of Risk Assessment Methodology to Genetically Engineered Food Products: A Generic Approach. Society for Risk Assessment. Abstract.

Frankos, V., M. Stedman, and M.A. Friedman. 1989. A lifetime oncogenicity study of acrylamide administered to F344 rats in the drinking water. Tenth Annual meeting of the American College of Toxicology. Program and Abstracts. p.26. Abstract.

Hanson, C.F., V.H. Frankos, and W.O. Thompson. 1989. Bioavailability of oxalic acid from spinach, sugar beet fibre and a solution of sodium oxalate consumed by female volunteers. Fd. Chem. Toxic. 27,3:181-184.

Frankos, V., L.H. Dulak, M.A. Fiedman. 1989. Use of risk assessment in the statistical design of a carcinogenicity bioassay of acrylamide. The Toxicologist 9:179. Abstract.

Strother, D.E., R.W. Mast, R.C. Kraska, and V. Frankos. 1988. Acrylonitrile as a carcinogen. Research needs for a better risk assessment. Annals of the New York Academy of Sciences 534:169-178.

Hanson, C.F., V.H. Frankos, and W.O. Thompson. 1988. Low dietary availability of oxalic acid present in refined sugar beet pulp compared to spinach and sodium oxalate. The Toxicologist 8:88. Abstract.

Strother, D.E., R.W. Mast, R.C. Krashka, and V. Frankos. 1988. Acrylonitrile as a Carcinogen: Research Needs for Better Risk Assessment, Annals of the New York Academy of Sciences 534:169-178.

Schwetz, A. Bernard, R.W. Tyl et al. 1987. Consensus workshop on the evaluation of maternal and developmental toxicity work group III report: Low dose extrapolation and other considerations for risk assessment - models and applications. Teratogenesis, Carcinogenesis, and Mutagenesis 7:321-327.

Frankos, V.H. and S.H. Rieth. 1987. Safety factors applied to various FDA pregnancy class drugs. The Toxicologist 7. Abstract.

Kimmel, C.A., G.L. Kimmel, and V. Frankos. 1986. Editors IRLG Workshop on Reproductive Toxicity Risk Assessment. *Environmental Health Perspectives,* Vol 66, 193-221.

Rodricks, V. Joseph, V. Frankos, D. Turnbull, R.G. Tardiff. 1986. Risk assessment for effects other than cancer. In Food Protection Technology. Proceedings of the 1986 Conference for Food Protection. Lewis Publishers, Inc.

Frankos, V.H. 1985. FDA perspectives on the use of teratology data for human risk assessment. *Fundamental and Applied Toxicology.* Vol. 5, 615-625.

Flamm, W.G. and V. Frankos. 1985. Formaldehyde: Laboratory Evidence. In "Interpretation of Negative Epidemiological Evidence for Carcinogenicity." IARC Scientific Publication 65.

Siegel, D.M., V.H. Frankos, and M.A. Schneiderman. 1983. Formaldehyde risk assessment for occupationally exposed workers. *Regulatory Tox. and Pharm.* 3, 355-371..

Frankos, V.H. 1982. Relationship of exposure to toxic chemicals and reproductive impairment. Expert testimony for Committee on Science and Technology, U.S. House of Representatives July 27, 1982. United State Government Printing Office.

Frankos, V.H. 1982. Qualitative comparison of chemical teratogenesis in humans and animal species (Abstract). Third Annual Meeting of the American College of Toxicology, Washington, D.C.

Siegel, D.M., V.H. Frankos, and M.A. Schneiderman. 1982. Formaldehyde risk assessment for occupationally exposed workers (Abstract). Third Annual Meeting of the American College of Toxicology, Washington, D.C.

Frankos, V.H. and J. Wassom. 1982. Computerized teratology literature data extraction, indexing, and collation (Abstract). *Teratology.* 25:2 80A.

Frankos, V.H. Symposium Summary and Suggested Future Directions for Detection of Neurotoxicity. 1980. In The Effects of Foods and Drugs on the Development and Function of the Nervous System: Methods for Predicting Toxicity. Edited by Gryder, R.M. and Frankos, V.H. U.S. Department of Health and Human Services, Food and Drug Administration, Publication No. DHHS/FDA 80-1076.

Frankos, V.H. 1980. Reproductive toxicity risk assessment task group: Outline of work plan and request for comments. Federal Register 45:63553-63554.

Gryder, R.M. and V.H. Frankos (eds.). 1980. The Effects of Foods and Drugs on the Development and Function of the Nervous System: Methods for Predicting Toxicity. U.S. Department of Health and Human Services, Food and Drug Administration.

Frankos, V.H. and R. Platt. 1976. Glycerol accumulation and water content in larvae of *Limenitis archippus*: Their importance to winter and survival. *J. Insect Physiol.* 22:623-623.

Frankos, V.H. and G. Butterbaugh. 1976. Characterization of norepinephrine metabolism following simultaneous intraventricular injection of H^3-L-tyrosine and C^{14}-DL-norepinephrine. *Pharmacologist* 18:135.

Messiha, K.F., E. Bakutis, and V.H. Frankos. 1973. Simultaneous separation of acid metabolites of catecholamines: Application to urine and tissue. *Clin. Chim. Acta.* 45:159-164.

ATTACHMENT 9

K-STATE
Kansas State University
Animal Sciences and Industry
K-State Research and Extension
232 Weber Hall
Manhattan, KS 66506-0201
785-532-6533
Fax: 785-532-7059

August 8, 2001

Eric F. Greenberg
Of Counsel
Ungaretti & Harris
3500 Three First National Plaza
Chicago, IL 60602-4283

Dear Mr. Greenberg:

The purpose of this letter is to confirm that I believe the use of a small quantity of carbon monoxide in the modified packaging system know as ActiveTech (by PACTIV) is safe and should qualify as GRAS. I have been a research meat scientist for nearly 30 years and have focused most of that time on factors affecting meat color and shelf life, including packaging systems. Thus, I am familiar with most of the world literature on such systems.

Based on my review of the details of the ActiveTech 2001 modified atmosphere system employing 0.4% carbon monoxide gas in a mixture with 60 percent carbon dioxide and the remainder nitrogen, as well as the published literature and common knowledge in the field, I am confident that the use of modified atmosphere including small quantities of carbon monoxide (0.4%) to package fresh meats as used in ActiveTech 2001 system is both safe and generally recognized as safe.

Sincerely,

Melvin C. Hunt

Melvin C. Hunt
Professor

MELVIN C. HUNT

Department of Animal Sciences and Industry
Weber Hall
Kansas State University
Manhattan, KS 66506-0201

Ph: 913-532-1232
Fax: 913-532-7059
Hhunt@oznet.ksu.edu

PERSONAL DATA:
Born: February 10, 1942
Married: Rae Jean Opie, August 20, 1965; Daughters: Paige and Holly

EDUCATION:
B.S. 1965 Animal Husbandry, Kansas State University, Manhattan, KS
M.S. 1970 Animal Science, Kansas State University, Manhattan, KS
Ph.D. 1973 Food Science, University of Missouri, Columbia, MO

PROFESSIONAL EXPERIENCE:
1991- Chair, Undergraduate Food Science Program
1984- Professor, Kansas State University: 50% Teaching - 50% Research
1978-84 Associate Professor, Kansas State University
1975-78 Assistant Professor, Kansas State University
1973-75 Research Chemist, Tennessee Eastman Company
1968-73 Grad Research Assistant, Kansas State and University of Missouri
1966-68 Taught high school chemistry and biology, Kinsley, KS

PROFESSIONAL AFFILIATIONS:

American Meat Science Association:
- President, 1995-96; Past-President, 1996-97
- Director and Executive Board, 1989-91
- Chair 1991 Reciprocal Meat Conference
- Parliamentarian
- Chair or member of numerous committees including:
  Meat Color Guidelines, AMSA Teaching Award, Undergraduate Travel Award, Grad Student Poster Competition, Teaching Display, Resolutions, Meat Tenderness, Biochemistry-Biophysics, Packaging, Meat Color, Growth and Development, Reciprocation, Long Range Planning, Sustaining Membership, Endowment, and Research Priorities.

American Society of Animal Science:
- Chair and Chair-elect, Meat Science-Muscle Biology Section of National ASAS Meeting
- Chair, Midwestern ASAS Meat Science Section
- Editorial Board Journal Animal Science
- Teaching Award Committee, Midwestern ASAS Section

Institute of Food Technologists:
- Chair and Chair-elect of Muscle Foods Division, 1992-94
- Director of Muscle Foods Division
- Chair of Muscle Foods Nominating Committee
- Committee for two National Muscle Foods Symposia
- Journal of Food Science, Manuscript Review

CAST: Contributing member

Journal of Muscle Foods: Editorial Board
Meat Science: Manuscript Review

HONORARY AFFILIATIONS:

Phi Kappa Phi, Sigma Xi, Phi Tau Sigma, Gamma Sigma Delta, Alpha Zeta

HONORS:

- College of Agriculture Outstanding Faculty Award 1979
- College of Agriculture Outstanding Faculty Award 1982
- College of Agriculture Outstanding Faculty Award 1988
- College of Agriculture Outstanding Faculty Award 1998
- College of Agriculture Outstanding Academic Advisor 1983
- University Selection for Parents' Day Lecture 1979
- Outstanding Lecturer Award, ITAL, Campinas, Brazil 1981
- Honorary State Farmer Degree 1985
- Distinguished Teaching Award, Gamma Sigma Delta 1989
- Selected Instructor, National Food Science Satellite Program 1990
- Certificate of Meritorious Service, Kansas Ag Teachers Association 1992
- CASE Professor of the year, Kansas winner of national competition 1992
- Outstanding Advising Award, Gamma Sigma Delta 1994
- Distinguished Teaching Award, American Meat Science Association 1994
- Outstanding Food Scientist, Phi Tau Sigma 1996
- Outstanding KSU Instructor & Advisor Award, Mortar Board 1997
- Signal Service Award, American Meat Science Association 1997
- USDA Food & Agriculture Science Excellence in Teaching Award, 2000

DEPARTMENT, COLLEGE OF AG, AND UNIVERSITY ACTIVITIES:

- Faculty Advisor: Block and Bridle, 6 years
- Faculty Advisor: Food Science Club, 3 years
- Faculty Advisor: Animal Science Grad Student Association, 16 years
- Faculty Advisor: Ag Student Council, elected for 2 terms (4 years)
- Chair, Weber Hall Building/Renovation Project
- Chair, KSU Meat Science Faculty
- Coordinator of KSU Meat Research Labs
- ASI Graduate Student Selection Committee
- ASI Undergraduate Career Development Committee
- ASI Library Committee
- ASI Scholarship, Loans and Honors Committee
- Department Representative for Gamma Sigma Delta, 10 years
- Student Team Coordinator, ASI Quadrathalon Teams
- Agriculture Student of the Month Selection Committee
- Agriculture Faculty of the Semester Selection Committee
- College of Agriculture Course and Curriculum Committee, chair and member
- College of Agriculture Academic Standards Committee, chair and member
- College of Agriculture Commencement Committee
- University Faculty Senator, College of Agriculture, two terms (6 years)
- University Academic Affairs Committee
- University Coordinating Committee for United Way
- KAES NCR-121 Chair and Secretary: Food & Feed Safety in Animal Production
- Food Science Undergraduate and Graduate Steering Committees
- Chair, Non-Traditional Studies Advisory Committee
- Elected by peers to ASI Teaching Advisory Committee
- Chair, KSU Undergraduate Food Science Program: Coordinate all course & curriculum and policy matters, scholarship, internships, recruitment, and record keeping

INDUSTRY-EXTENSION ACTIVITIES:

- Numerous presentations at: MidWest Meat Processors Seminars, Kansas-Nebraska Curing and Sausage Short Courses, KSU Cattlemen's Day, KSU Swine Day
- Technical Assistance for: Tennessee Eastman Company, Ross Industries, Giant Food

Stores, Excel Corporation, IBP, Doskocil Companies, Tenneco Packaging, Farmland, National Beef, Cryovac, Buckhead Beef, Dupont, Kalsec, Wendy's, Greater Omaha Beef, Hormel
- State FFA Livestock Awards Selection Committee
- State FFA Star Farmer Selection Committee
- State FFA Public Speaking Contest Judge
- Kansas Jr. Livestock Carcass Contest Judge
- Kansas Meat Processor Cured Meat Show Judge
- Missouri Meat Processor Cured Meat Show Judge

TEACHING RESPONSIBILITIES:

Current Courses - KSU Campus:

- ASI 350 Meat Science. 3hr. Lecture-lab introductory meat science
  Enrollment: Since 1979, 2031 students; currently running at maximum seating of 72
- ASI 610 Processed Meat Operations. 2hr. 50% responsibility, value-added processing
  Enrollment: 6 to 12 undergraduate and graduate students; since 1988, 35 students
- ASI 930 Advanced Meat Science. 3hr. Team-taught, highest level meats course
  Enrollment: Varies from 6 to 15 graduate students
- GENAG 500 Food Science Seminar. 1hr. Seminar for graduating seniors
  Enrollment: Varies from 6 to 15 students

Current Courses - KSU Distance Learning Program:

- ASI 340 Principles of Meat Science. 2hr. Web-based course for Continuing Education
  Enrollment: Since 1987, over 680 students
- GENAG 500 Food Science Seminar. 1hr. Seminar series for Distance Learning majors
  Enrollment: 3 to 15 undergraduate students per year, Continuing Education
- GENAG 630 Food Science Problems. 1hr. Detailed written investigation of current topics
  Enrollment: 2 to 8 students per year through Continuing Education

Previously Taught Courses:

- Topics in Meat Science and Muscle Biology
- Meats Judging Team (at University of Missouri)
- Meat Processing
- Livestock and Meat Evaluation
- Animal Agriculture and Consumers

INTERNATIONAL COURSE ACTIVITIES:

- Meat Science and Technology Short Course for Latin America, Institute for Food Technology, Campinas, Brazil, 6 weeks, one of two international lecturers
- Meat Science Facilities, University of Monterrey, Monterrey, Mexico
- Lecturer for five KSU International Meat Science Courses, International Meat and Livestock Program, Kansas State University
- Sabbatical leave, fall 1992, visiting scientist to Norwegian Food Research Institute, As
- Have attended 8 International Congresses of Meat Science and Technology

ADVISING RESPONSIBILITIES:

- Undergraduate Advisees: average of 26 for the last 10 years
- Graduate Students Supervised:
  - 12 Masters Students
  - 6 PhD Students
- Graduate Student Committees:
  - 43 Masters
  - 20 PhD
- Coordinate student-company relations for employment and internships for FSI

RESEARCH INTERESTS:

- Myoglobin chemistry and meat color, Methods of color measurement, Cooked meat color and food safety, Postmortem factors affecting meat quality, Collagen chemistry, Low-fat ground beef and processed meats; Six major company packaging projects funded since 1994 dealing with shelf life, color life, cold chain management, product palatability, and microbiology

PUBLIC AND COMMUNITY ACTIVITIES:

- Manhattan Optimist Club: committees for many youth activities
- Coach, Girls (16-18) ASA fast pitch softball traveling team
- Executive Committee, Riley County Extension Council
- Asst. Superintendent, sheep division, Riley County Fair
- Judge at Manhattan High School oratorical contest
- FarmHouse Fraternity, alumni board and committee work
- Snyder Award for Alumni Service, FarmHouse Fraternity
- Activities of First Presbyterian Church

Melvin C. Hunt
Professor
Department of Animal Sciences and Industry
Kansas State University

Refereed Journal Articles

Hunt, M.C., R.A. Smith, D.H. Kropf and H.J. Tuma. 1975. Factors affecting showcase color stability of frozen lamb in transparent film. J. Food Sci. 40:637.

Hunt, M.C. and H.B. Hedrick. 1977. Profile of fiber types and related properties of five bovine Muscles. J. Food Sci. 42:513.

Hunt, M.C. and H.B. Hedrick. 1977. Histochemical and histological characteristics of bovine muscle from four quality groups. J. Food Sci. 42:578.

Hunt, M.C. and H.B. Hedrick. 1977. Chemical, physical and sensory characteristics of bovine muscle from four quality groups. J. Food Sci. 42:716.

Thomas, J.D., D.M. Allen, M.C. Hunt and C.L. Kastner. 1977. Nutritional regimen, post-slaughter conditioning temperature, and vacuum packaging effects on beef carcass and retail cut bacteriology. J. Food Prot. 40:678.

Harrison, A.R., M.E. Smith, D.M. Allen, M.C. Hunt, C.L. Kastner and D.H. Kropf. 1978. Nutritional regimen effects on quality and yield characteristics of beef. J. Anim. Sci. 47:383.

Loveday, H.D., M.E. Dikeman, M.C. Hunt and A.D. Dayton. 1978. Adipose tissue water related to bovine carcass composition. J. Anim. Sci. 47:606.

Smith, M.E., C.L. Kastner, M.C. Hunt, D.H. Kropf and D.M. Allen. 1979. Elevated conditioning temperature effects on carcasses from four nutritional regimens. J. Food Sci. 44:158.

Gutowski, G.H., M.C. Hunt, C.L. Kastner, D.H. Kropf and D.M. Allen. 1979. Vacuum aging, display, and level of nutrition effects on beef quality. J. Food Sci. 44:140.

Fung, D.Y.C., C.L. Kastner, M.C. Hunt, M.E. Dikeman and D.H. Kropf. 1980. Mesophilic and psychrotrophic populations on hot-boned and conventionally processed beef. J. Food Prot. 43:547.

Hayward, L.H., M.C. Hunt, C.L. Kastner and D.H. Kropf. 1980. Blade tenderization effects on beef longissimus sensory and Instron textural measurements. J. Food Sci. 45:925.

Harrison, A.R., D.H. Kropf, D.M. Allen, M.C. Hunt and C.L. Kastner. 1980. Relationships of spectrophotometric reflectance measurements to beef muscle visual color. J. Food Sci. 45:1052.

Burson, D.E., M.C. Hunt, D.M. Allen, C.L. Kastner and D.H. Kropf. 1980. Ration energy density and time on feed effects on beef longissimus palatability. J. Anim. Sci. 51:875.

Fung, D.Y.C., C.L. Kastner, C-Y. Lee, M.C. Hunt, M.E. Dikeman and D.H. Kropf. 1981. Initial chilling rate effects on bacterial growth of hot-boned beef. J. Food Prot. 44:539.

Wu, J.J., D.M. Allen, M.C. Hunt, C.L. Kastner and D.H. Kropf. 1981. Nutritional effects on beef collagen characteristics and palatability. J. Anim. Sci. 53:1256.

Hall, J.B. and M.C. Hunt. 1982. Collagen solubility of A-maturity bovine longissimus muscle as affected by nutritional regimen. J. Anim. Sci. 55:321.

Sieper, P.S., M.C. Hunt, D.H. Kropf, C.L. Kastner and M.E. Dikeman. 1983. Electrical stimulation effects on myoglobin properties of bovine longissimus muscle. J. Food Sci. 48:479.

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Hunt, M.C. 1999. Distance learning methods. KSU College of Agriculture Focus Series.

Educational Materials

Course Syllabi:

Distance Learning - Complete course, *PRINCIPLES OF MEAT SCIENCE* in "any-time or real-time" distance learning format via audiotapes, teleconferencing, on-line discussions involving collaborative learning, problem solving and critical thinking via the Internet.

In-Class
- Laboratory Materials for *MEAT SCIENCE*, a series of 14 study guides for lab exercises
- Laboratory Materials for *PROCESSED MEAT OPERATIONS*

Other Materials and Activities:

USDA Grant:
- Expanding Undergraduate Education for Food Industry Personnel via Technology. 1994-96 USDA Challenge Grant Program, $79,479

Web-based Course - Principles of Meat Science, KSU Division of Continuing Education

Color Guides
- Ground Beef Patty Cooked Color Guide
- Cured Meat Color Guide
- Cooked Pork Chop Color Guide
- Ground Pork Patty Cooked Color Guide

Science Series - Lesson Plans for: Promoting Ag Science for Secondary Schools
Developing New Meat Products
Color Chemistry in Meat Products
Meat Packaging Exercises for High School Students

Slides Series:
- Unraveling the Mystery of Premature Browning in Cooked Ground Beef Patties
- Doneness of Cooked Ground Beef
- Dynamics of Conversion of Myoglobin Forms
- Role of Pigment Layers in Influencing Surface Meat Color
- Spray Chilling of Carcasses
- Don't be Broken-Hearted because of High-fat in Ground Beef
- Commercial Sausage, Ham and Bacon Production
- Food Science at KSU
- ASI Quadrathlon - why I should participate
- Updated: Muscle-Bone Anatomy; Beef-Pork-Lamb Cut Identification

Video Tapes:
- Beef Carcass Electrical Stimulation and Hot Boning (Edited with M. E. Dikeman)

Store Survey: - Out-of-class assignment for Analysis of Retail Meat Section of Grocery Stores

Diet Survey: - Out-of-class assignment for computerized class project of Nutritional Value of Muscle Foods in the student's diet

Current topic: Survey - Out-of-class assignment for critically analyzing printed literature on a variety of livestock and meat industry topics

Web Sites: - Out-of-class assignment for evaluation and collection of scientific facts about muscle biology and meat science

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Hayward, L.H. 1978. Blade tenderization effects on subjective and instron objective textural measurements of longissimus steaks from cattle fed various nutritional regimes. M.S. Thesis, Kansas State University.

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Highfill, G.A. 1984. Effects of sex and compudose implantation on porcine muscle histochemistry. M.S. Thesis, Kansas State University.

Burson, D.E. 1985. Effects of muscle, heat and sex on the proportions of types I and III bovine intramuscular collagen. Ph.D. Dissertation. Kansas State University.

Claus, J.R. 1989. Characterization of low-fat processed meat containing dietary fiber. Ph.D. Dissertation, Kansas State University.

Troutt, S.E. 1990. A chemical, physical and sensory characterization of low-fat ground beef. M.S. Thesis, Kansas State University.

Warren, K.E. 1990. Modified atmosphere packaging with 100% carbon dioxide for bone-in pork loins. M.S. Thesis, Kansas State University.

Hague, M.A. 1992. Internal and expressible juice color of cooked ground beef patties. M.S. Thesis, Kansas State University.

Londono Villegas, J.F. 1993. Effects of realimentation and trenbolone acetate implantation of cull cows on tenderness and cooked color. M.S. Thesis, Kansas State University.

Payne, A.C. 1993. Starch functionality and modification for batter sausages. PhD. Dissertation, Kansas State University.

Warren, K.E. 1994. Factors affecting premature browning of heated myoglobin. PhD. Dissertation, Kansas State University.

Conner, J.G. 1995. Fat trimming and vitamin E effects on subprimal and retail cut yields and shelf life. M.S. Thesis, Kansas State University.

Schoenbeck, J.J. 1997. Effects of carcass infusion on color and display life of several bovine muscles. M.S. Thesis, Kansas State University.

Clark, T.J. 1998. Rebloom and display color stability of beef and pork packaged in an ultra-low oxygen modified atmosphere active packaging system. M.S. Thesis, Kansas State University.

Campbell, R.E. 2000. Effects of internal structure, patty shrinkage, and desinewing on palatability attributes of ground beef. PhD dissertation.

Sammel, L.M. 2000. Chemical characterization, color stability, and comparison of assays for metmyoglobin reducing ability in beef inside and outside semimembranosus. MS thesis.

Lien, R. 2001. Effects of endpoint temperature, pork quality, and cooking factors on internal cooked color of pork chops and patties. MS thesis.

Wendelburg, J. 2001. Physical, chemical and microbial qualities of blade tenderized prime rib. MS Thesis.

Ballard, C. 2002. Carbon monoxide in modified atmosphere packaging. MS Thesis.

ATTACHMENT 10

CURRICULUM VITAE

Navn/Name:	Sørheim, Oddvin
Privatadresse/Private address:	Lyngveien 24A, 1430 Ås
E-mail:	oddvin.sorheim@matforsk.no
Telefon/Telephone:	+47-64970100
Arbeidssted/Place of employment:	MATFORSK – Norwegian Food Research Institute Osloveien 1, 1430 Ås
Stilling/Present position:	Sjefingeniør / Senior Research Technologist Dr.agric. / Ph.D.

Utdanning/Education:

2000, Dr. agric./Ph.D., Norges landbrukshøgskole/Agricultural University of Norway

1982, Cand. agric./M.Sc., Næringsmiddelfag, Norges landbrukshøgskole/Agricultural University of Norway

Erfaring/Experience:

01.05. 1985 til nå ved/ to present at MATFORSK, arbeidet med forskning og industrioppdrag på kjøtt i hovedsak innen gasspakking, farge, mørhet, salting og funksjonelle egenskaper/ mainly working in research and industry consulting on packaging, color, tenderness, salting and functional properties of meat.

01.08. 1989 til 01.06. 1990, forskningsopphold/visiting scientist, Kansas State University, Department of Animal Sciences and Industry, Manhattan, KS, USA

01.06. 1982 til 30.04. 1985, produksjonskonsulent/consultant, Slakterienes Salgssentral/Norwegian Meat Cooperative, Oslo

Vitenskapelige originalarbeider/Scientific publications:

- Sørheim, O., Idland, J., Halvorsen, E.C., Frøystein, T., Lea, P., Hildrum, K.I. 2001. Influence of beef carcass stretching and chilling rate on the tenderness of M. longissimus dorsi. Meat Science, Vol 57, pp 79-85.
- Kjos, N.P., Øverland, M., Bryhni, E.A., Sørheim, O. 2000. Food waste products in diets for growing-finishing pigs: effect on growth performance, carcass characteristics and meat quality. Acta Agric. Scand., Sect. A, Animal Sci., Vol 50, pp 193-204.
- Hunt, M.C., Sørheim, O., Slinde, E. 1999. Color and heat denaturation of myoglobin forms in ground beef. Journal of Food Science, Vol 64, pp 847-851.
- Sørheim, O., Nissen, H., Nesbakken, T. 1999. The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. Meat Science, Vol 52, pp 157-164.
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Vitenskapelige publikasjoner og presentasjoner/Other publications and presentations:

- Sørheim, O. 2000. Effects of modified atmosphere packaging on colour and microbiological shelf life of red meats. (Dr.agric./Ph.D.-thesis, Agricultural University of Norway).
- Sørheim, O., Nissen, H. 2000. Current technology for meat MAP. Int. Food Marketing & Technology, Vol 14, No 4, pp 39-42.
- Sørheim, O., Nissen, H. 1996. Modified atmosphere packaging of red meats. The European Food & Drink Review, No 4, pp 77-80.

Bok/book

- Hildrum, K.I., Dainty, R.H., Egelandsdal, B., Larssen, E.G., Mielnik, J., Sørheim, O. 1996. Meat for the Consumer. MATFORSK Ås, 593 pp, ISBN 82-90394-58-6.

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- Risvik, E., Hildrum, K.I., Russwurm jr, H., Dainty, R.H., Egelandsdal, B., Green, A., Larssen, E.G., Merok, K.J., Mielnik, J., Norang, R.E., Næs, H., Olsen, M.Ø., Roskifte, P., Slinde, E., Sørheim, O. 1996. The 42nd ICoMST: "Meat for the Consumer", Norway 1-6 September 1996 (organising committee).
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